FRACTURE RESISTANCE OF CALCIUM HYDROXIDE-TREATED TEETH FILLED WITH WATER- AND PBS-MIXED MTA

A DISSERTATION PRESENTED FOR THE DEGREE OF

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LIST OF ABBREVIATIONS

Al	Aluminium
Bi	Bismuth
CA	Citric acid
Ca	Calcium
Ca^{2+}	Calcium ion
CH	Calcium hydroxide
EBA	Ethoxy benzoic acid
EDTA	Ethylenediaminetetraacetic acid
EDX	Energy Dispersive X-ray
EPMA	Electron Probe Micro-analyzer
F-max	Maximum force at fracture
IF	Interfacial
O	Oxygen
OH^-	Hydroxyl ion
MPa	Mega Pascal
MTA	Mineral Trioxide Aggregate
MTA(PBS)	MTA mixed with Ca- and Mg-free PBS
MTA(W)	MTA mixed with water
µm	Micrometer
N	Newton
NaOCl	Sodium hypochlorite
P	Phosphorus
P	P value

PBS	Phosphate-buffered saline
PDL	Periodontal ligament
SD	Standard deviation
SEM	Scanning electron microscope
Si	Silicon
STF	Synthetic tissue fluid
TDI	Traumatic dental injuries
WMTA	White MTA
VRF	Vertical root fracture
VS	Versus
2/52	Dressed for 2 weeks
12/52	Dressed for 12 weeks

LIST OF APPENDICES

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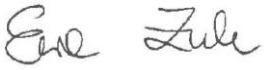
DECLARATION

I, Ewa Zuk, declare that the following dissertation in entirely my own work.

Signed:



Date:

20-07-2016

CERTIFICATE

I hereby certify that this candidate has fulfilled the condition of Ordinance Number 12 and the Regulations of the University of Dundee for the Degree of Master of Dental Science.

Signed by: A Jordan Chadwick

Date: 19th September 2016

ABSTRACT

Background: Traumatic dental injuries and dental caries are the main reasons why permanent teeth with immature, thin-walled roots lose their vitality and require endodontic treatment. Currently there are three endodontic treatment options to treat teeth with irreversibly damaged dental pulps and incomplete root formation. These are: pulp preservation, pulp regeneration and apical barrier induction with calcium hydroxide (CH) or production by placement of calcium silicate materials e.g. Mineral Trioxide Aggregate (MTA) in the apical part of the root canal.

MTA apexification can be undertaken as a one-visit treatment or two-visit treatment with a short-term CH pre-medication. The majority of *in vitro* studies on influence of CH on fracture strength showed reduction in the mechanical properties of radicular dentine after 5 weeks exposure (Yassen & Platt 2013). MTA may increase the resistance to vertical root fracture of endodntically treated teeth treated in one-visit (EL-Ma'aita et al. 2014).

Aim: To test, *in vitro*, the fracture strength of extracted human teeth restored with 5 mm apical plugs of MTA mixed with either water or Ca- and Mg-free Phosphate Buffered Saline (PBS), to establish whether these materials strengthen the root, and if an initial CH dressing has any effect on the fracture strength.

Materials and methods: One hundred and eighty freshly extracted human permanent single-rooted teeth were used. The crowns of these teeth were removed and 10 mm long roots were prepared until a Largo Peeso drill of a

size 150 ISO could be inserted 1 mm beyond the apical root-end, to resemble thin-walled incompletely formed roots. The roots were randomly allocated to nine groups (n=20). All samples were irrigated with equal volume of 5.25% sodium hypochlorite and 10% citric acid using a standardised protocol. Group Irrigation only - control group, received irrigation only; Group 2/52 CH - control group, dressed with calcium CH for 2 weeks; Group 12/52 CH - control group, dressed with CH for 12 weeks; Group MTA(W) - filled with water-mixed MTA (MTA-W); Group 2/52 CH + MTA(W) - dressed with CH for 2 weeks and filled with MTA-W; Group 12/52 CH + MTA(W) - dressed with CH for 12 weeks and filled with MTA-W; Group MTA(PBS) - filled with PBS-mixed MTA (MTA-PBS); Group 2/52 CH + MTA(PBS) - dressed with CH for 2 weeks and filled with MTA-PBS; Group 12/52 CH + MTA(PBS) - dressed with CH for 12 weeks and filled with MTA-PBS. All samples were stored for 4 weeks at 37ºC and 100% humidity.

Following the storage period, 18 samples from each group were mounted in acrylic resin, with simulated periodontal ligament using a polyether impression material. The roots were subjected to a compressive force using an Instron® Universal testing machine until fracture, and the maximum force at fracture (F-max) were recorded in Newtons (N). A metal jig was designed and fabricated for this purpose which permitted the prepared tooth specimens to be loaded by the tip of a chisel at 130º to the long axis of the tooth in a lingual-labial direction. The fracture type and depth were recorded. The interface between the material and the tooth structure of the remaining 2 samples from each group underwent element mapping using a Scanning Electron Microscope (SEM) with an Energy-dispersive X-ray detector.

Several images of the dentine and the dentine-MTA interface were taken using the SEM.

Results: Fracture resistance data were analyzed statistically by one-way ANOVA and the Tukey's comparison of means tests. The mean F-max (± SD) were: 462.34 ± 205.42 for Group Irrigation only; 598.9 ± 194.67 for Group 2/52 CH; 706.56 ± 240.66 for Group 12/52 CH; 920.41 ± 403.05 for Group MTA(W); 446.68 ± 201.07 for Group 2/52 CH + MTA(W); 409.17 ± 211.52 for Group 12/52 CH + MTA(W); 852.61 ± 375.7 for Group MTA(PBS); 832.36 ± 328.73 for Group 2/52 CH + MTA(PBS); 513.87 ± 272.44 for Group 12/52 CH + MTA(PBS).

Significant differences ($P < 0.05$) in fracture resistance were found between groups: 12/52 CH +MTA(W) vs 12/52 CH, 2/52 CH + MTA(PBS), MTA(PBS), MTA(W); 2/52 CH + MTA(W) vs 2/52 CH + MTA(PBS), MTA(PBS), MTA(W); Irrigation only vs 2/52 CH + MTA(PBS), MTA(PBS), MTA(W); 12/52 CH + MTA(PBS) vs 2/52 CH + MTA(PBS), MTA(PBS), MTA(W); and 2/52 CH vs MTA(W). The Weibull moduli analysis flagged up, missed by the Tukey analysis of fracture strength data, issues concerning higher dependability of groups 12/52 CH vs 2/52 CH + MTA(W) and 12/52 CH + MTA(PBS); and 2/52 CH + MTA(PBS) vs 2/52 CH. The analysis showed also poorer dependability of samples in group MTA(W) vs 2/52 CH + MTA(PBS) and MTA(PBS).

The results of Chi-square calculation for two types of fracture: split and comminuted (Chi-square = 19.06, $P = 0.015$) and three depths of fracture: above the cylinder, into the cylinder and vertical root fracture (Chi-square = 50.46, $P = 0.00002$) rejected the null hypotheses that the experimental

groups and fracture types and depths are independent. In the SEM examination of groups filled with MTA mixed with PBS, it was possible to distinguish the interfacial layer between the cement and dentine, and a 50-200 μm layer of altered dentine adjacent to MTA. No such findings could be noted in groups filled with MTA mixed with water.

Conclusions: This study, with its limitations, has confirmed that: 1) CH dressing for up to 12 weeks had no negative effect on fracture resistance of human roots; 2) There is no significant change in fracture resistance of human teeth that received a 2- and 12-week treatment with CH and were restored with MTA-W, or treated with CH for 12 weeks, and restored with MTA-PBS, in comparison with teeth that received no treatment; 3) One-visit apexification with MTA mixed with either water or Ca- and Mg-free PBS can significantly improve fracture resistance of human teeth. One-visit apexification with MTA mixed with Ca- and Mg-free PBS is the more dependable treatment option to strengthen thin-walled teeth; 4) If CH dressing is required for disinfection of a thin-walled tooth before apexification with MTA, it is better to use it short-term (2 weeks) and obturate the root canal with MTA mixed with Ca- and Mg-free PBS. This will have a root strengthening effect on the human teeth; 5) Obturation with MTA apical plug has negative effect on fracture resistance of human roots treated with CH for extended periods (12 weeks); 6) The fracture force has no influence on the type or depth of root fracture in teeth treated with either CH or MTA apexification. The type of apexification treatment may have an influence on the depth and the type of root fracture; 7) MTA mixed with PBS can produce an interfacial layer between the cement and the dentine even

in teeth that received pre-treatment with CH; 8) The element diffusion from MTA into dentine is possible when MTA is mixed with PBS, even if pre-treatment with CH was used. When MTA is mixed with water, CH pre-medication seemed to prevent the element migration to dentine.

CHAPTER ONE: INTRODUCTION

In young permanent teeth with immature roots, Hertwig's sheath, together with a vital pulp are necessary to continue apexogenesis (root formation; a histological term used to describe the continued physiological development and formation of the apex of the root) and form a tooth with a favourable crown/root ratio and a root that is thick enough to withstand normal function.

The endodontic treatment of teeth with immature root formation has always been a challenge because of the large diameter apices that makes working length determination and subsequent obturation difficult.

The aim of this study is to test, *in vitro*, the fracture strength of extracted human teeth with apical plugs of Mineral Trioxide Aggregate mixed with either water or Ca- and Mg-free Phosphate Buffered Saline, and to establish whether these materials strengthen the root. An analysis will be made of the interface between the material and the tooth structure using a Scanning Electron Microscope equipped with an Energy Dispersive X-ray detector to observe potential mineral exchange at this interface.

CHAPTER TWO: LITERATURE REVIEW

2.1. REASONS FOR ENDODONTIC TREATMENT OF IMMATURE TEETH

Traumatic dental injuries (TDI) and dental caries are the main reasons why permanent teeth with immature roots lose their vitality.

In many patients that have suffered dental trauma or caries it is possible, through appropriate treatment, to maintain the vitality of the pulp of a tooth. Unfortunately, often the damage to the pulp is too serious, or the treatment is undertaken too late to preserve vitality of the pulp. A tooth with an irreversibly inflamed or necrotic pulp, appropriately treated, can remain clinically functional, often for years. Craniofacial growth, which can continue up to the age of 25 years (Heij et al. 2006) can be completed uninterrupted. Even if the tooth is lost after this time, more definitive treatment options, such as an implant-supported crowns or fixed prostheses are available to the patient.

2.1.1. Traumatic Dental Injuries

The overall prevalence of dental injuries in the UK was reported to be of the order of 15% overall, and 34-44% in deprived areas (Hamilton et al. 1997). According to others one in five children had experienced a TDI to their permanent anterior teeth before leaving school (O'Brien M. 1994). Outwith the UK two large national surveys in the USA indicated that approximately one in six adolescents showed evidence of a TDI (Shulman & Peterson 2004, Kaste et al. 1996).

Luxation injuries appear to be associated with the greatest risk of incomplete root development with between 15% and 59% of teeth losing their vitality. The maxillary central incisors are the most frequently affected teeth (Andreason et al. 2011).

In the case of luxation injuries, the trauma can rupture the neurovascular supply at the level of the apical foramen, whereas in horizontal root fractures, the rupture can occur at the level of fracture. A disruption of the blood supply of the tooth can cause tissue asphyxia, which will lead to inflammation, and irreversible pulpitis, which will eventually lead to liquefaction pulp necrosis (Andreasen et al. 1990). Pulpitis following trauma can initiate the resorption of adjacent hard tissues, which can make endodontic treatment more challenging, and affect adversely its prognosis. In all trauma-induced (non-infective) tooth resorption some damage to the cementum-periodontal membrane complex has occurred which stimulates osteoclastic activity (Heithersay 2007).

The prevalence of TDI has grown to alarming levels and several factors have been identified (Glendor 2009) (Table 2.1) that increase the risk for TDI:

- Oral (increased overjet with protrusion),
- Environmental (deprived areas),
- Behavioural (risk-taking children, children being bullied, emotionally stressful conditions, obesity and attention-deficit hyperactivity disorder),
- Other (presence of illness, learning difficulties, physical limitations, tongue piercing, amateur sports athletes and inappropriate dental habits).

References	Region	Year	Age/age group (years)	Physical leisure activity	Collision	Fall	Sport	Traffic accident	Violence	Inappropriate use of teeth or biting a hard item	Other	Unknown
	Asia											
Uji and Teramoto	Japan	1988	6–18	–	–	37.7	29.2	1.6	7.9	–	23.6	–
Chen et al.	Central Taiwan	1999	Mean 8.2	–	65.3	26.9	3.6	–	2.6	–	1.6	–
	Europe											
Blinkhorn	UK	2000	11–14	18.5	–	33.9	17.2	14.6	4.3	–	–	11.5
	Middle East											
Baghdady et al.	Iraq	1981	6–12	–	–	54.0	3.0	2.4	35.8	–	–	4.9
Baghdady et al.	Sudan	1981	6–12	–	–	18.3	3.3	2.8	70.6	–	–	5.0
Marcenes et al.	Syria	1999	9–12	–	16.0	9.1	–	24.1	42.5	–	3.4	4.6
	South America											
Garćia-Godoy et al.	Dom Rep.	1981	7–14	–	1.7	50.0	–	5.1	–	–	10.2	32.4
Garćia-Godoy	Dom Rep.	1984	5–14	36.6	–	–	49.4	14.0	–	–	–	–
Marcenes et al.	Brazil	2000	12	–	6.8	26.0	19.2	20.6	16.4	–	9.6	1.4
Nicolau et al.	Brazil	2001	13	–	15.0	24.1	2.3	10.5	1.5	6.0	–	40.6
Traebert et al.	Brazil	2003	12	–	37.5	47.9	–	2.1	–	2.1	–	10.4
Soriano et al.	Brazil	2007	12	9.1	18.2	27.3	8.2	2.7	6.4	1.8	3.6	22.7

Table 2.1. Frequency of causes (in per cent) of traumatic dental injuries presented according to region of study (Glendor 2009)**.**

2.1.2. Dental caries

The National Dental Inspection Programme held in Scotland in the school year 2014/2015, reported that 24.7% of examined 12-year old children (Primary 7) had dental caries experience in their permanent teeth.

The Children's Dental Health Survey undertaken in England, Wales and Northern Ireland in 2013 reported that 46% of 15-year old and 34% of 12 year old children had "obvious decay experience" in their permanent teeth.

An American study on Virginia schoolchildren showed that 25% to 65% of children had untreated caries (Brickhouse et al. 2007).

Deep caries induces severe inflammatory reactions in the pulp and may cause pulp necrosis. The majority of permanent teeth in children in the age group 8-10 years have incomplete formation of the root apex, and some of those teeth will require endodontic treatment in the form of a partial pulpotomy or root canal treatment if the caries involves the pulp.

The response of the periradicular tissues to pulp necrosis alone, or superimposed on trauma-induced resorption, is inflammation, which may result in apical periodontitis often combined with tooth root resorption (Heithersay 2007).

2.2. ENDODONTIC TREATMENT OPTIONS FOR TEETH WITH IMMATURE ROOTS

Currently there are three endodontic treatment options to treat teeth with irreversibly damaged dental pulps and incomplete root formation. These are:

- Pulp preservation
- Pulp regeneration
- Apical barrier induction with calcium hydroxide (CH) or production by placement of calcium silicate materials e.g. Mineral Trioxide Aggregate (MTA) in the apical part of the root canal.

Regenerative endodontic procedures can be defined as “biologically based procedures designed to predictably replace damaged, diseased, or missing structures, including dentine and root structures, as well as cells of the pulp-dentine complex, with live viable tissues, preferably of the same origin, that restore the normal physiological functions of the pulp-dentine complex” (Murray et al. 2007).These procedures can include: direct pulp capping, revascularization, apexogenesis, apexification, stem cell therapy, and tissue engineering (Murray et al. 2007).

2.2.1. Pulp preservation

The aim of deep pulpotomy (Cvek's pulpotomy) is to maintain pulp vitality and function, and is recommended in teeth with coronal pulp damage and no apical pathology, such as teeth with mechanical or recent traumatic pulp exposure. The coronal, irreversibly inflamed pulp is amputated and dressed with a capping material, usually CH or MTA. As part of the wound healing a new generation of odontoblast-like cells is formed, reconstructing the lost continuum at the pulp-dentine border.

A systematic review of vital pulp therapy in vital permanent teeth with cariously exposed pulp has shown that a partial pulpotomy has a high success rate of 99.4% up to more than 3 years. Teeth with wide apices showed a less successful outcome than those with closed apices (90.6% vs 94.6%) (Aguilar & Linsuwanont 2011).

2.2.2. Pulp regeneration

Revascularization is a regenerative treatment that is biologically based and designed to allow the continuation of root development. When successful, revascularisation can be associated with significantly greater increases in root length and thickness, compared with CH or MTA apexification techniques (Jeeruphan et al. 2012).

Regeneration can be achieved through the activity of cells from the pulp, periodontium, vascular or immune systems or stem cell therapies. Most of the current therapies involve the use of the host's own pulp or vascular cells and are based on the process of revascularisation (Garcia-Godoy & Murray 2012). The majority of such treatments may best be described as under development indicating they are not ready for widespread clinical application.

Such treatments produce an ingrowth of bone into the pulp space with cementum forming an inner root surface, so in consequence there is no real regeneration of pulp.

There are numerous case reports and case series in the literature reporting successful outcomes of revascularization in the treatment of immature permanent teeth with necrotic pulps with and without apical periodontitis (Garcia-Godoy & Murray 2012). There are no standard treatment protocols and a lack of long-term evidence to support the use of regenerative endodontic procedures in treatment of teeth with immature apices. This technique is currently recommended if the tooth is not suitable for root canal obturation, and only after attempts at apexification with calcium hydroxide or partial pulpotomy treatments have been unsuccessful (Garcia-Godoy & Murray 2012).

2.2.3. Apexification with calcium hydroxide

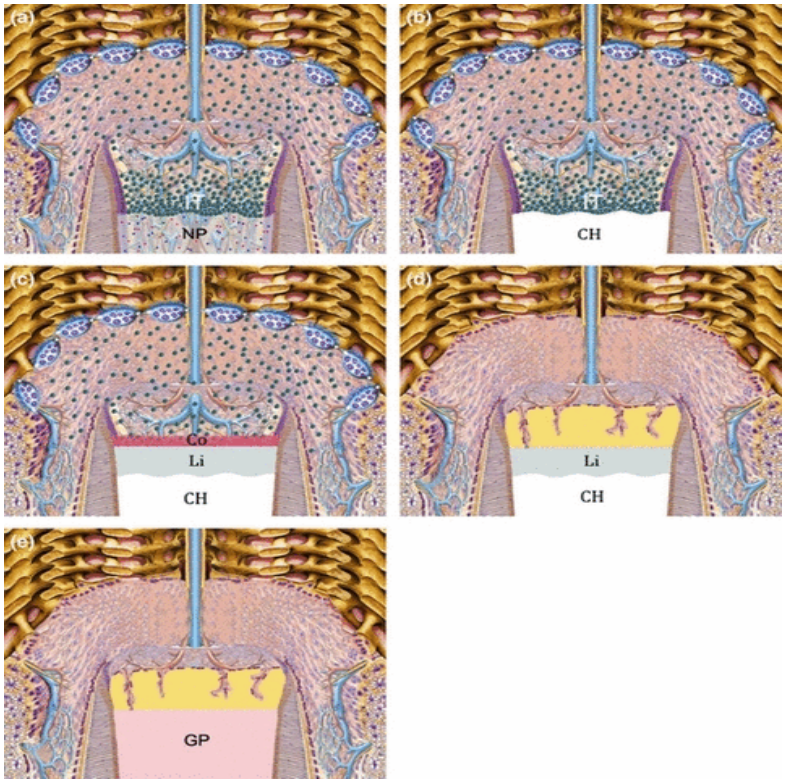
Apexification (root-end closure), is a method of inducing root-end closure of an incompletely formed non-vital permanent tooth, by placement of CH paste in the root canal space.

CH stimulates the formation of a hard (osteoid or cementoid) tissue barrier across the open apical foramen. The main purpose of the hard tissue barrier is to prevent overextension of the root filling materials into the apical tissues (Mackie et al. 1988). These obturating materials were, until recently, not biocompatible. The barrier provides a matrix against which the root filling material can be placed, reduces the surface area of the root filling in contact with the periapical tissues thereby reducing the exposure of the sealer to the tissue fluids. The rate at which the sealer can be dissolved is also reduced and provides a better barrier to leakage.

The exact mechanism of hard tissue formation is not completely understood, through its high pH, CH initiates formation of zones of liquefaction and coagulation necrosis of the adjacent vital periapical tissues. The necrotic layer then calcifies passively (Heithersay 1970), usually as a cementum-like structure (Figure 2.1). It is postulated that calcium hydroxide activates alkaline phosphatase and stimulates undifferentiated mesenchymal cells to differentiate into cementoblasts and osteoblasts, allowing hard tissue formation. To optimise success it is important that the CH is placed at the apex as a solid barrier.

In the course of apexification, continued root formation can be seen on most occasions; the common appearance is that of a dome-shaped “cap” around the apex. This hard tissue bridge is permeable, irregular, layered, and has numerous vascular channels which perforate it. This could lead to bacterial invasion from the infected pulp space through these channels to the periapical tissues (Cvek & Sundstrom 1974). A dense acellular cementum-like tissue forms the outer layer. More centrally located, dense and irregular fibrocollagenous connective tissue with granular inclusions of foreign material and irregular fragments of calcification can be found (Baldassari-Cruz et al. 1998).

The apexification procedure was first reported by Granath (Granath 1959) in 1959, and widely popularised by Frank (Frank 1966) and Heithersay (Heithersay 1970). Apexification with CH is a relatively simple and predictable procedure with reported success rates between 74% (Thater & Marechaux 1988) and 100% (Heithersay 1970, Cvek 1972), with an average of over 95% (Bakland & Andreasen 2012) (Table 2.2).



(a) Infected pulp necrosis (NP) plus inflammation (IT) in the apical part of the root canal. (b) Dressing with calcium hydroxide (CH). (c) Because of its high pH effect upon dentine, calcium hydroxide (CH) causes the release of a number of wound healing signals (growth factors), and for some time, the high pH also may prevent bacteria from entering the wound healing site. CH induces by its high pH effect apical liquefaction (Li) and a coagulation zones (Co) of necrosis. (d). The response to the coagulation necrosis appears to be recruitment of new hard tissue forming cells from the apical tissues, these are usually of cementoblastic origin, but may also be osteoblasts. During this process, vascular inclusions may occur. After 6-18 months, a hard tissue barrier is formed. (e) Status after root filling with gutta-percha (GP).

Figure 2.1. Calcium hydroxide induced apexification changes after pulp necrosis (Bakland & Andreasen 2012)**.**

Examiner	Number of teeth	Healing (%)
Kerekes et al. (Kerekes et al. 1980)	66	62 (94)
Vojinovic (Vojinovic 1981)	100	98 (98)
Mackie et al. (Mackie et al. 1988)	112	108 (96)
Yates (Yates 1988)	48	37 (77)
Merglova (Merglova 2001)	33	31 (94)
Cvek (Cvek 1992)	328	314 (96)
Total	687	650 (95)

Numbers not parenthesised are actual tooth numbers. Parenthesised numbers are % of sample.

Table 2.2. Periapical healing following initial treatment with calcium hydroxide and subsequent gutta-percha root filling in teeth with pulp necrosis and immature roots (Bakland & Andreasen 2012).

The adverse events and long-term effects of CH apexification lacks evidence (Rafter 2005). There is not only no clinical evidence that its use may help avoid root fracture (Garcia-Godoy & Murray 2012), but the extended use of CH in this technique has been linked with an increased risk of cervical root fracture (Størmer et al. 1988, Cvek 1992).

Some debate exists as to whether the CH dressing needs to be replaced and if so at what intervals this should be done. There are several protocols used for the procedure. Some endodontists believe that the CH is only required to initiate the healing reaction and it is sufficient to place the CH dressing once

only and wait for radiographic evidence of apexification (Cvek 1972, Chala et al. 2011). In such an approach the dressing should be replaced only when symptoms develop, or when the dressing has washed out of the apical two-thirds of the root canal. However, if symptoms do occur then the healing process will be delayed because of the presence of infection. The lack of contact of the CH with the apical tissues may also delay healing (Yates 1988).

Others, (Mackie et al.1988, Mackie et al. 1994, Abbott 1998) prefer to use several regular applications of fresh CH. Regular CH dressing replacements are recommended (Abbott 1998) to:

- Maintain a high pH within the root canal to ensure an anti-bacterial environment is maintained,
- Allow regular and sustained delivery of hydroxyl ions to the periapical tissues which helps to promote hard tissue repair,
- Ensure complete contact between the calcium hydroxide and the apical tissues,
- Replace the temporary restorations as they often lose their marginal seal,
- Assess the progress of the barrier formation clinically with paper points ideally using a dental operating microscope. The barrier forms from the periphery towards the centre and is difficult to assess radiographically,
- Replace the CH paste which washes out of the canal readily, especially in the early stages of apexification,

- Reduce exposure to unnecessary radiation; radiographs only show gross changes so a considerable amount of hard tissue must be formed before it is visible on a radiograph, a much longer treatment time may be used than is necessary. Radiographs cannot be relied upon as they are only a two dimensional view of an object. CH pastes have a similar radiopacity to dentine and the apical tissue barrier and may be difficult to distinguish on a radiograph.

Therefore, CH apexification is a time-consuming treatment, it can take on average 5.1 +/- 4.5 months and 2.4 +/- 1.5 visits (Mackie et al. 1988), and up to 18 months to reach completion. Whilst several recalls are advised, treatment success may be jeopardized if patients fail to attend (Mackie et al. 1988) or because of a breakdown of the temporary restoration leading to re-infection of the root canal system.

2.2.4. Apexification with Mineral Trioxide Aggregate

Mineral Trioxide Aggregate apexification, may not fall within the classic definition of apexification, as there is no calcified barrier or continued root development prior to an obturation of the apical part of the root canal with MTA. Biological apical closure appears later, after filling of the root canal, contrary to the calcium hydroxide apexification technique, where obtaining the apical barrier is necessary to complete the root canal treatment.

Shabahang and Torabinejad first described the use of MTA as an apical barrier in teeth with open apices (Shabahang & Torabinejad 2000). It has been shown that MTA behaves in a similar way to CH paste when in contact with connective tissues promoting protein denaturation and necrosis by coagulation (Yaltirik et al. 2004). MTA can stimulate repair because it allows cellular adhesion, growth and proliferation on its surface (Zhu et al. 2000) and has the ability to induce hard tissue formation when used adjacent to the periradicular tissues (Shabahang et al. 1999) (Figure 2.2).

Currently, two treatment protocols exist for MTA apexification. These are:

- two-visit treatment, with a short-term dressing of CH for canal disinfection, followed by the placement of an MTA apical barrier (Shabahang et al. 1999, Sarris et al. 2008).
- one-visit apexification with MTA without the use of CH (Steinig et al. 2003, Simon et al. 2007).

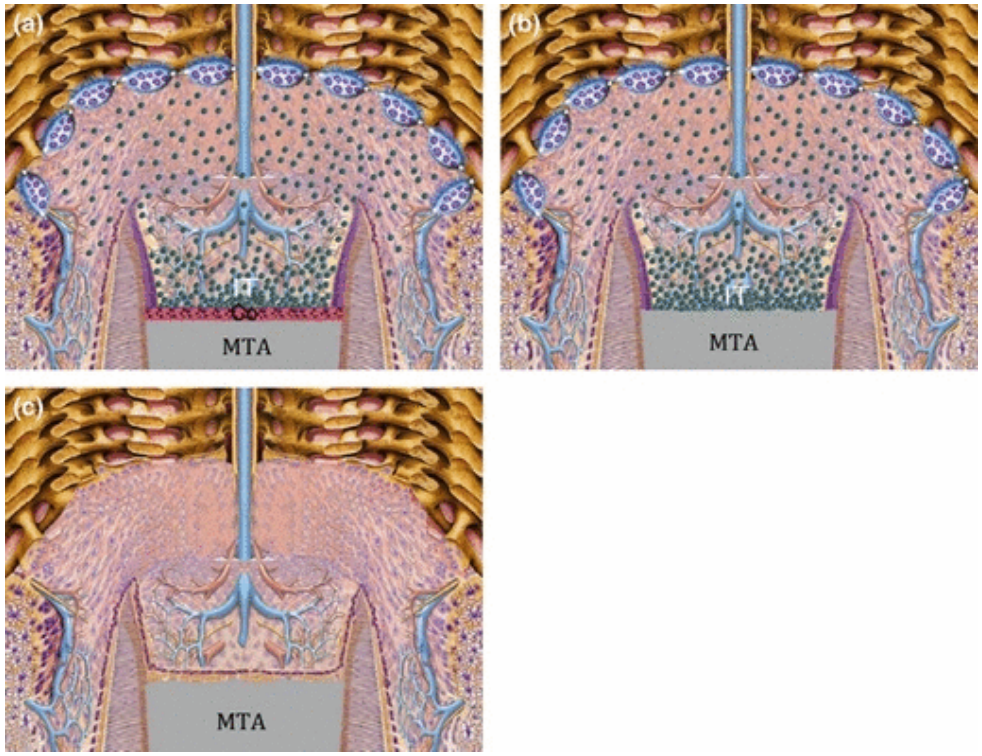
The techniques show radiographic success rates of 89% (Bakland & Andreasen 2012) (Table 2.3).

The advantages of the MTA apexification technique may be summarised as:

- Reduction in treatment time and number of visits
- Possibility to restore the tooth with minimal delay and prevent the risk of re-infection and tooth fracture
- Avoids changes in dentine mechanical properties caused by prolonged use of CH
- Successful in teeth with resorption (Guzeler et al. 2010).

The disadvantages of the MTA apexification technique can be summarised as:

- Both versions of the MTA, white and grey have a potential to discolour teeth.
- Once solidified, the MTA is very difficult to remove from straight root canals and impossible to remove from curved root canals.
- Slow “setting” time may be seen as a relative disadvantage, but it allows for radiographic quality control and re-placement of the material if required.
- The obturation of the entire root canal with MTA is expensive, but current consensus is to place 4-5 mm MTA as an apical barrier (Lawley et al. 2004, Al-Kahtani et al. 2005), with the remainder of the canal obturated with gutta-percha and a sealer.



(a) Mineral trioxide aggregate (MTA) induces, by its high pH effect upon dentine, a release of wound healing signals (growth factors). Subsequent to this a physical bond between MTA and dentin provides a barrier against bacterial penetration. MTA induces by its high pH effect a very narrow zone of coagulation necrosis (Co). Next to that zone, a reparative cementum zone is formed. (c) Subsequently, the hard tissue barrier is associated with formation of normal PDL attachment to the cementum layer.

Figure 2.2. Mineral trioxide aggregate induced apexification changes after pulp necrosis (Bakland & Andreasen 2012)**.**

Examiner	No of teeth	Healing (%)
El-Meligy and Avery (El-Meligy & Avery 2006)	15	15 (100)
Pradhan *et al.* (Pradhan et al. 2006)	10	10 (100)
Pace *et al.* (Pace et al. 2007)	11	10 (91)
Simon *et al.*(Simon et al. 2007)	57	46 (81)
Sarris *et al.* (Sarris et al. 2008)	17	13 (77)
Holden *et al.* (Holden et al. 2008)	20	17 (85)
Witherspoon *et al.* (Witherspoon et al. 2008)	116	106 (92)
Moore *et al.* (Moore et al. 2011)	22	21 (96)
Total	268	238 (89)

Numbers not parenthesised are actual tooth numbers. Parenthesised numbers are % of sample.

Table 2.3. Periapical healing following treatment with MTA in teeth with pulp necrosis and immature roots (Bakland & Andreasen 2012)**.**

2.2.5. Is a short-term CH dressing necessary prior to MTA obturation?

Felippe *et al.* (Felippe et al. 2006) showed, using an *in vivo* model in dogs infected immature teeth, that apical calcified barriers were formed in all specimens, whether they were obturated immediately with MTA or after dressing for 1 week with CH paste. The initial use of CH has been shown to be strongly related to the extrusion of MTA and formation of barriers beyond the limits of the root canal walls. The authors hypothesized that sodium hypochlorite (NaOCl) together with CH dressing could cause necrosis and tissue dissolution of the periapical tissues leading to extrusion of the MTA.

In a clinical study, fifty-seven human teeth with open apices received an apexification procedure in one appointment with MTA. At 12 months healing occurred in 81% of cases (Simon et al. 2007).

A study comparing 1-vist and 2-visit (with 3-week dressing with CH) MTA apexification, showed high success rates after at least 12 months, and no difference between the groups (93.5% in 1-visit vs 90.5% in 2-visit group) (Witherspoon et al. 2008).They recommended that MTA should be placed in one visit.

2.3. CALCIUM HYDROXIDE

2.3.1 Chemical and physical properties of calcium hydroxide

Since its introduction in 1920 by Hermann (Hermann 1920), CH has been widely used in endodontics because of its antimicrobial, tissue dissolving, anti-resorptive and hard tissue-forming properties. For over six decades, it has been the main material used for apexification.

Bacterial invasion of the dental pulp often leads to the need for root canal treatment, and as healing of the periapical tissues and the formation of a hard tissue barrier will not occur in the presence of bacteria, it is essential to eliminate all the microorganisms in the root canal system.

CH is a white odourless powder with the chemical formula $Ca(OH)_2$. It has low solubility in water, which decreases as the temperature rises. CH is used to disinfect the root canal and prevent bacterial ingress.

2.3.2. Mode of action of calcium hydroxide

CH is a strong alkaline substance, with a pH of approximately 12.5. In aqueous solution, CH dissociates into calcium (Ca^{2+}) and hydroxyl (OH^-) ions. Hydroxyl ions are highly oxidant free radicals that show extreme reactivity, reacting with several biomolecules.

Three mechanisms of OH^- lethal action on bacterial cells have been described in the literature (Siqueira & Lopes 1999), these are:

- Induction of lipid peroxidation, resulting in the destruction of the cellular membrane
- Induction of the breakdown of ionic bonds that maintain the tertiary structure of proteins that results in the loss of biological activity of enzymes and disruption of the cellular metabolism
- Reacting with the bacterial DNA, inhibiting DNA replication and inducing lethal mutations

CH can also act as a physicochemical barrier, preventing both the proliferation of residual microorganisms and the re-infection of the root canal by bacteria from the oral cavity (Siqueira Jr & de Uzeda 1997).

Through its antibacterial properties CH kills microorganisms, and prevents their ingress into the root canal system. The medicament acts both as a physical barrier and bacteriocide by withholding substrates for growth and limiting space for bacterial multiplication (Slots et al. 1992). The high pH of CH creates an environment that promotes periapical tissue healing (Heithersay 1970).

2.3.3. Antimicrobial properties of calcium hydroxide

Several bacterial species commonly found in infected root canals are eliminated after a short period when in direct contact with CH. The lethal effect of CH on bacterial cells is only observed when the substance is in direct contact with bacteria in solution, and the concentration of hydroxyl ions is very high (Bystrom et al. 1985). Clinically, this direct contact and high pH are not always possible to achieve. pH values have been shown to be decreased in the more distant parts of the root canal, and at different depths in the dentine because of the buffering capacity of dentine (Tronstad et al. 1981).

The presence of a CH dressing for one week raised the pH of the inner dentine to approximately 9.0 (Tronstad et al. 1981, Nerwich et al. 1993). As shown by Padan *et al.* (Padan et al. 1981) the majority of bacteria grow well within the pH range of 6-9, but some *Enteroccocci* can tolerate pH values of 9-11. This may be due to the activation of specific proton pumps, specific enzymatic and/or buffering devices within the biofilm, or by the production of bacterial by-products (Padan et al. 1981). The biofilm type of bacterial growth, in which the cells located at the periphery of colonies can protect those located more deeply inside the dentinal tubules, may play a role (Siqueira Jr & de Uzeda 1996).

When Ca^{2+} comes into contact with carbon dioxide or carbonate ions in tissue, calcium carbonate is formed which alters the mineralization process

by the overall consumption of Ca^{2+} ions. Calcium carbonate has neither biological nor antibacterial properties (Estrela et al. 1999).

Kim and Kim in their two-part literature review of the antimicrobial effect of CH both *in vivo* (Kim & Kim 2014) and *in vitro* (Kim & Kim 2015) studies concluded that the antimicrobial effects of CH showed varied and often conflicting results. Some studies supported the antimicrobial efficacy of CH, whereas others questioned it. They concluded that the addition of vehicles or other agents such as chlorhexidine, might contribute to the antimicrobial effect of CH. The use of CH as an intracanal medicament represented better histological results in animal studies (Table 2.4). However, human clinical studies showed limited antimicrobial effects with some microorganisms reduced but not eliminated through the treatment, and some species with a resistance to CH (Tables 2.5 and 2.6). Most of the clinical outcome studies they reviewed supported that there is no improvement in healing of the periapical lesions when CH was applied between appointments (Table 2.7).

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Year	Researcher	Test method (animal)	Major ingredient	Period	Result
1983	Stevens & Grossman	Culture (cat)	CH solution, slurry, Pulpdent, CMCP	21 day	CH solution: ineffective CH slurry, Pulpdent: limited effect
1999	Katebzadeh *et al*.	Histopathology (dog)	CH	1 wk (sacrificed 6 mon)	CH: less inflammation
2000	Katebzadeh *et al*.	Radiograph (dog)	CH	1 wk (x-ray 6 mon)	CH: fewer failed cases more improved cases
2002	Leonardo *et al*.	Histopathology (dog)	CH + CMCP		30 day: Better results
2002	Tanomaru Filho *et al.*	Histopathology (dog)	CH + CMCP	15 day (sacrificed 210 day)	Better results than immediate obturation
2005	De Rossi *et al*.	Radiograph (dog)	CH + CHX	15 day (x-ray 30, 75 and 120 day)	120 day: Reduction of lesion size

CH = Calcium hydroxide; CMCP = Camphorated paramonochlorophenol; CHX = Chlorhexidine.

Table 2.4. Animal studies on the antimicrobial effect of CH (Kim & Kim 2015)**.**

Year	Researcher	Major ingredient	Period	Effect
1985	Byström *et al*.	CH, CP, CMCP	1 mon	+
1985	Safavi *et al*.	CH, IKI		+
1991	Sjögren *et al*.	CH	1 wk	+
1997	Barbosa *et al*.	CH, CMCP, CHX	1 wk	+
1999	Molander *et al*.	CH	2 mon	+/-
2000	Shuping *et al*.	CH	7 - 200 day	+
2002	Peters *et al*.	CH	1 mon	+/-
2005	Zerella *et al*.	CH, CHX, CH+CHX	7 - 10 day	+/-
2006	Chu *et al*.	CH, Ledermix, Septomixine	1 wk	+/-
2006	Oncag *et al*.	CH, CHX, CH+CHX	2 day	+/-
2007	Manzur *et al*.	CH, CHX, CH+CHX	1 wk	-
2007	Vianna *et al*.	CH, CHX, CH+CHX	1 wk	-
2013	Sinha *et al*.	CH, CHX, CH+CHX	1 wk	+/-

+/- = the result showed a limited effect; CH = Calcium hydroxide; CP = Camphorated phenol; CMCP = Camphorated paramonochlorophenol; IKI = Iodine potassium iodide; CHX = Chlorhexidine.

Table 2.5. Human clinical studies on the antimicrobial effect of CH by bacterial culture (Kim, Kim 2015)**.**

Year	Researcher	Major ingredient	Period	Effect
2004	Tang *et al*.	CH, Septomixine	1 wk	-
2005	de Souza *et al*.	CH	2 wk	+/-
2007	Sakamoto *et al*.	CH + CMCP	1 wk	-
2007	Siqueira Jr *et al*.	CH	1 wk	-
2007	Siqueira *et al*.	CH + CMCP	1 wk	+
2007	Siqueira *et al*.	CH + CHX	1 wk	+
2010	Rocas *et al*.	CH + CMCP	1 wk	+/-
2011	Ito *et al*.	CH + CHX	2 wk	+/-
2011	Rocas *et al*.	CH, CH + CMCP	1 wk	+/-
2013	Paiva *et al*.	CH + CHX	1 wk	+/-

+/- = the result showed a limited effect; CH =Calcium hydroxide; CP = Camphorated phenol; CMCP = Camphorated paramonochlorophenol; IKI = Iodine potassium iodide; CHX = Chlorhexidine.

Table 2.6. Human clinical studies on the antimicrobial effect of CH by molecular methods (Kim & Kim 2015)**.**

Year	Researcher	Test method	Interappointment period	Follow-up period	Result
1999	Trope *et al*.	Radiograph	1 wk	1 yr	+
2000	Weiger *et al*.	Radiograph	7 – 47 day	5 yr	-
2002	Peters and Wesselink	Radiograph	4 wk	4.5 yr	-
2005	Waltimo *et al*.	Radiograph	1 wk	1 yr	-
2007	Molander *et al*.	Radiograph	1 wk	2 yr	-

+ = The use of CH resulted better outcome; - = The results did not show significant difference.

Table 2.7. Clinical outcome studies on the use of CH as an intracanal medicaments (Kim & Kim 2015)**.**

2.3.4. Anti-endotoxin activity of calcium hydroxide

Endotoxins (lipopolysaccharides) are the major constituent of the outer cell wall of Gram negative bacteria, mostly Bacteroides species. They are secreted in vesicles by growing bacteria and released after bacterial death. Endotoxins have been detected in 100% of primary and secondary endodontic infections. They elicit strong immune responses in the periradicular tissues. High levels of endotoxin are linked to the severity of bone destruction and development of symptoms (Gomes et al. 2012). A review of the anti-endotoxin activity of CH demonstrates that CH has ability to inactivate bacterial lipopolysaccharides (Mohammadi et al. 2012).

Marinho *et al.* (Marinho et al. 2014) demonstrated the effectiveness of the chemo-mechanical preparation in the removal of endotoxin from root canals of teeth with chronic apical periodontitis. Moreover, in this study the use of intracanal medication of CH for 30 days contributed to an improvement in endotoxin reduction.

2.3.5. Influence of vehicles on calcium hydroxide properties

A CH paste for use in endodontics is composed of the powder, a vehicle and a radiopacifier. Other substances are added to the paste to maintain consistency, improve flow and radiopacity and maintain high pH in clinical use (Fava & Saunders 1999).

The vehicle plays the most important role in the process of CH dissociation because it determines the velocity of ionic dissociation causing the paste to be solubilised and resorbed at various rates by the periapical tissue. It also determines the frequency of intracanal dressing replacements that should be provided. Three types of CH vehicle are used in endodontics: aqueous, viscous and oily (Fava & Saunders 1999).

When CH is mixed with one of the aqueous solutions (e.g. water, saline, Ringer's solution, aqueous suspension of methylcellulose or carboxymethylcellulose), the Ca^{2+} and OH^- ions are rapidly released. This type of vehicle promotes a high degree of solubility when the paste remains in contact with the tissue and tissue fluids, causing it to be rapidly solubilised and resorbed by macrophages. The root canal may become empty in a short period (Fava & Saunders 1999).

Viscous vehicles (e.g. glycerine, polyethyleneglycol, propylene glycol) are also water soluble, but release Ca^{2+} and OH^- ions in a more gradual and uniform fashion and for extended periods. The high molecular weight of those vehicles minimizes the dispersion of CH into the tissue, maintains the paste in the desired area for longer, and prolongs the CH action (Fava & Saunders 1999).

Oily vehicles are non-water-soluble substances (such as camphor, olive oil, silicone oil and some fatty acids) that promote the lowest solubility and diffusion of the paste within the tissues. Oily vehicle pastes have limited application (Fava & Saunders 1999).

2.3.6. Synergistic effect of calcium hydroxide and sodium hypochlorite on tissue dissolution

In wide root canals of immature teeth, some tissue debris may remain after initial instrumentation has been carried out. CH can dissolve those tissues directly or indirectly, by making pulp tissue more readily dissolved by NaOCl (Andersen et al. 1992). CH and NaOCl have been shown to work synergistically on pulp tissue dissolution and dentine disinfection (Zehnder et al. 2003).

2.4. STRUCTURE AND MECHANICAL PROPERTIES OF DENTINE

2.4.1. STRUCTURE OF DENTINE

Mature dentine is a composite material made up of an organic fraction (30 wt%), which is mainly collagen and an inter-penetrant inorganic fraction (60 wt%) and water (10 wt%).

The inorganic phase in dentine is mainly composed of poorly crystalline carbonated hydroxyapatite with a needle and/or plate like morphology (10x 50 nm), which exists both within the collagen fibrils (intra-fibrillarly mineralized) and between fibrils (inter-fibrillarly mineralized) on a nanometric scale.

Ninety percent of the organic phase is collagen, which is exclusively Type I. This type of collagen is a strong, three-dimensional fibrous polymer that usually exists in an aqueous biological environment. It is often associated with proteoglycans, which contains a large amount of bound water.

There are two forms of water in dentine. One is associated with the apatite crystal of the inorganic phase, and collageneous and non-collageneous matrix proteins of the organic phase. This water is ‘tightly’ bound. The second is the free or ‘unbound’ water, and fills the dentinal tubules and other porosities in the dentine matrix. The free water is associated with inorganic ions such as calcium and phosphate, and aids in their transport within the dentine matrix. Free water can be removed by heating at 100°C, but bound

water can only be substantially removed by heating at 600°C (Van Der Graaf & Ten Bosch 1990).

2.4.2. Mechanical properties of dentine

The main determinant of stiffness in dental hard tissue is the mineral concentration in the tissue. During mineralization, when more and more mineral component displaces water, the hard tissue becomes stiffer (Currey 2002). High levels of mineralization result in high values of elastic modulus and static strength, while also resulting in more brittle tissue behaviour. The toughness and/or increased strain energy is provided by the organic fraction, particularly the collagen in dentine. The degree of mineralization of the collagen substrate in dentine varies continuously with location, and this gradient in the mineralization is believed to optimize the functionality of the overall tooth structure.

The role of the different constituents on the mechanical characteristics of dentine is shown in Figure 2.3.

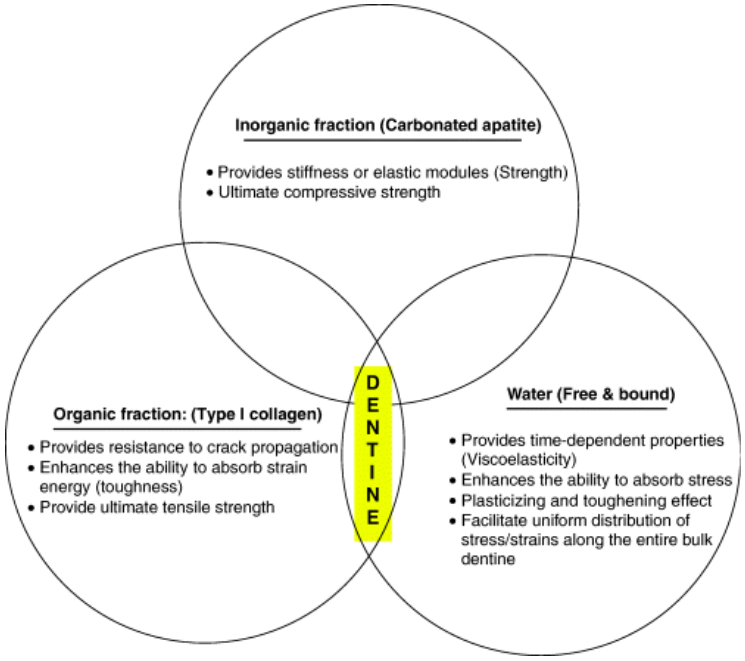


Figure 2.3. The role of different constituents on the mechanical integrity of structural dentine (Kishen 2006)**.**

2.4.3. Risk factors for root fracture

The causes of fracture of endodontically treated teeth have been extensively discussed by Kishen (Kishen 2006), and are summarised in Figure 2.4. The risk factors can be classified as iatrogenic and non-iatrogenic. CH dressing, MTA and endodontic irrigants can be considered as iatrogenic causes of teeth fracture.

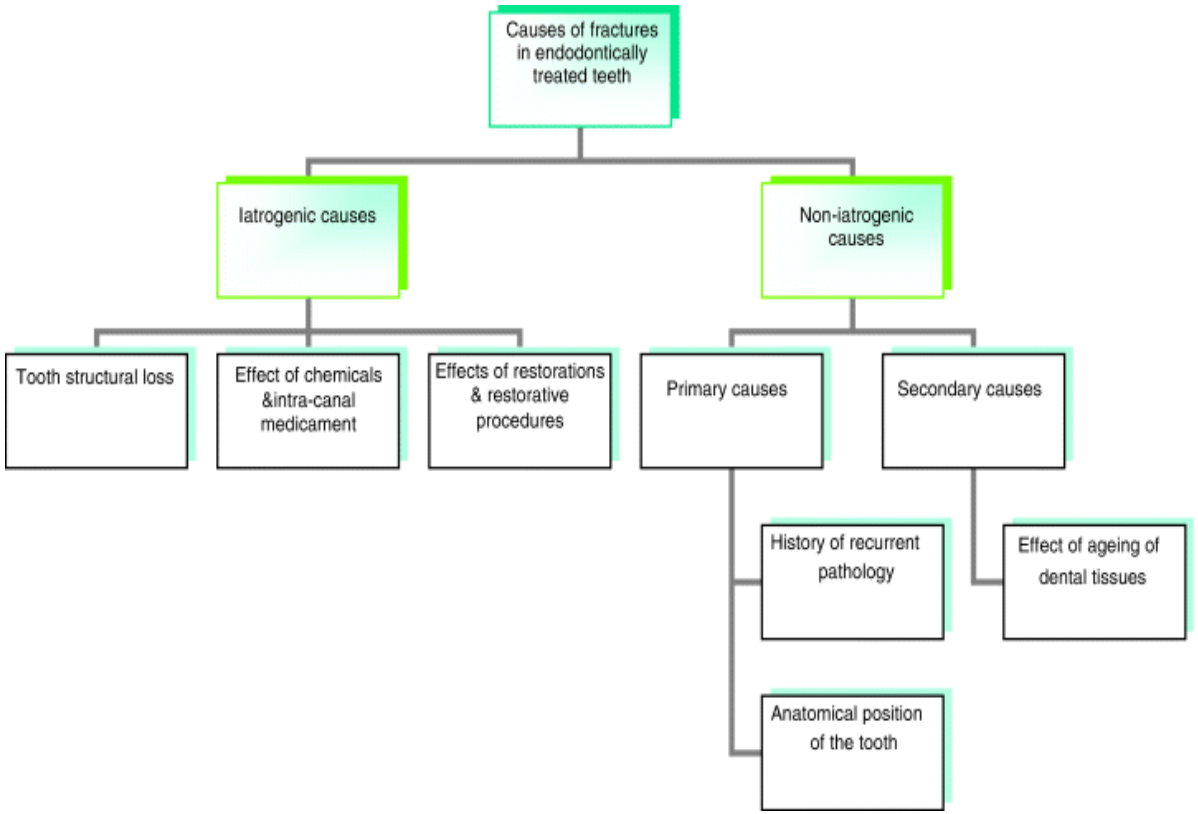


Figure 2.4. Outline of the causes of fracture in endodontically treated teeth (Kishen 2006)**.**

2.4.4. Influence of sodium hypochlorite and chelating agents on dentine strength

The current endodontic disinfectant regimen involves the use of 0.5% - 6% sodium hypochlorite for irrigation throughout the procedure, followed with final irrigation with chelating agents: either 10%-40% citric acid (CA) or 17% - 18% ethylenediaminetetraacetic acid (EDTA) and sodium hypochlorite before placement of a CH intracanal dressing.

The adverse effects of NaOCl on physical properties such as flexural strength, elastic modulus, and microhardness of the dentine have been reported (Grigoratos et al. 2001, Goldsmith et al. 2002). These changes in the physical properties of dentine arise due to the changes in the inorganic and the organic phases of the dentine.

Temperature, time, and concentration have all been shown to contribute to the penetration of NaOCl into dentinal tubules. The highest penetration (300 μm) was obtained with 6% NaOCl for 20 minutes at 45ºC (Zou et al. 2010). When chelating agents were used after NaOCl for the final irrigation no additional erosion was noted. However, if NaOCl irrigation followed the application of a chelating agent, more erosion resulted (Qian et al. 2011). The authors concluded that, when NaOCl was used in a procedure, the hydroxyapatite coating on the collagen fibrils seemed to protect them, thus limiting dentine erosion. In contrast when a chelator was used it removed the hydroxyapatite layer and exposed the collagen fibrils, thus facilitating a direct

attack upon the now exposed collagen fibrils producing dentine erosion in a relatively short time.

Grigoratos *et al.* (Grigoratos et al. 2001) investigated the effect of NaOCl and CH on the flexural strength and modulus of elasticity of dentine bars submerges in either NaOCl or CH for 2 hr or 1 week . Their results have showed that both NaOCl and CH reduce the flexural strength of dentine. NaOCl also reduced the modulus of elasticity of dentine whereas CH did not. Sequential use of NaOCl and CH had no additional weakening effect.

2.5. CALCIUM HYDROXIDE APEXIFICATION AS A RISK OF ROOT FRACTURE

In 1988, a disturbing observation was presented by Størmer *et al.* (Størmer et al. 1988) at a paedodontic meeting in Norway, claiming that 60% of all endodontically treated teeth with immature root formation had cervical fractures due to minor impacts, with even some spontaneous fractures. Similar findings were published by Cvek in 1992 (Cvek 1992), whose 4-year retrospective clinical study reported a cervical root fracture rate of 40% amongst 885 luxated, nonvital, immature incisors treated with long-term CH intracanal dressing. In comparison, root fractures of permanent teeth are uncommon, their incidence ranges from 0.5% to 7.0% for all cases of dental trauma (Andreasen et al. 1989).

2.5.1. Effect of calcium hydroxide on dentine

Some (Andreasen et al. 2002, White et al. 2002) have postulated that the high pH of CH could lead to neutralization, dissolution and denaturation of acidic proteins and other proteoglycans in dentine, which may affect the linking of the collagen fibrils to the hydroxyapatite crystals. Others (Kawamoto et al. 2008) are of the opinion that the alkalinity of CH might lead to the breakdown of the inorganic dentine structure or the denaturing of the collagen network, resulting in poorer fracture resistance. Because the

collagen fibrils are not readily accessible to CH, time would be required for CH to penetrate into and denature the collagen fibrils for such an effect to occur.

A study by Tronstad *et al*. (Tronstad et al. 1981) demonstrated that there was a pH gradient in the roots of monkey teeth 1 month after placement of CH dressing, with higher values around the canal compared with more peripheral sites.

2.5.2. Studies on influence of calcium hydroxide on fracture strength

In their classic paper Andreasen *et al.* (Andreasen et al. 2002) examined the fracture strength of immature mandibular incisors from sheep. The teeth were divided into two experimental groups:

- Group 1: the pulps were extirpated via the apical foramen, the root canals were then filled with CH and sealed with Intermediate Restorative Material (IRM), and the teeth were then stored in saline at room temperature for 0.5, 1, 2, 3, 6, 9, or 12 months.
- Group 2: the pulps were extirpated and the root canals were filled with saline and sealed with IRM cement. The teeth were then stored in saline at room temperature for 2 months.
- Intact teeth served as controls and were tested immediately after extraction.

They found the fracture strength of sheep dentine was reduced after 60 days of CH application (Andreasen et al. 2002).

In a recent systematic literature review, Yassen and Platt (Yassen & Platt 2013) reviewed articles published between 1953 and 2012, and included at least one experimental group with root or root dentine filled or exposed to CH, and one control group and a minimum of five samples per experimental group. They found no clinical studies that directly supported the correlation between CH intracanal dressing and root fracture in the literature. The majority of *in vitro* studies showed reduction in the mechanical properties of radicular dentine after exposure to CH for 5 weeks or longer. Conversely, the data were inconclusive regarding whether CH exposure for 1 month or less had a negative effect on the mechanical properties of radicular dentine (Table 2.8). None of the included studies incorporated a contemporary endodontic irrigation protocol.

A study evaluated *in vivo* the fracture resistance of human dentine exposed to CH for 15, 30 and 60 days in comparison to control group that received no treatment, using the edge chipping method. All calcium hydroxide exposed test groups showed statistically greater edge toughness values relative to the control group. Chip resistance may reflect both the fracture resistance and the hardness of dentine, a quasi brittle material (Whitbeck et al. 2011).

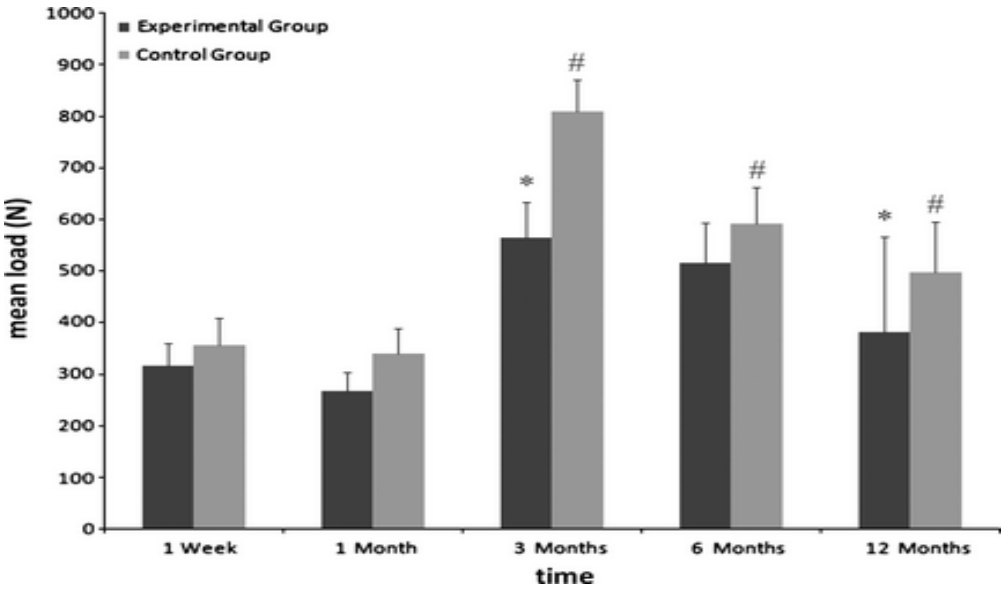
In another study (Zarei et al. 2013) one hundred single-rooted premolar human teeth were divided into two groups, one group received no treatment, whereas the other group received treatment with CH. Teeth were stored for 1 week, or 1, 3, 6, or 12 months at 37ºC and 100% humidity. The periodontal

ligament was simulated using a layer of wax. The teeth then underwent fracture resistance testing. The mean compressive force required to fracture teeth at various time periods is presented in Figure 2.5. The study results showed that the mean maximum force at fracture was significantly higher in the control group at 1, 3, and 6 months. But, interestingly, the mean compressive force required to fracture those teeth seemed to increase with time in both groups, and more than doubled at 3 months, compared to week one.

Authors (year)	Type of teeth used	Tested mechanical properties as reported in the studies	Mechanical test used	Duration of exposure	Pertinent outcomes
a) Animal studies					
Andreasen *et al*. (Andreasen et al. 2002)	Sheep	Fracture strength	Load to failure	0.5,1, 2, 3, 6, 9, or 12 months	The fracture strength of $Ca(OH)_2$-filled teeth was significantly reduced after 2, 3, 6, 9 and 12 months
White *et al*. (White et al. 2002)	Bovine	Fracture strength	Shear stress	5 weeks	$Ca(OH)_2$ exposure significantly reduced the fracture force
Andreasen *et al*. (Andreasen et al. 2006)	Sheep	Fracture strength	Load to failure	100 days	The fracture strength of $Ca(OH)_2$-filled teeth was significantly reduced
Hatibović-Kofman*et et al*. (Hatibovic-Kofman et al. 2008)	Sheep	Fracture resistance	Load to failure	2 weeks, 2 months, and 1 year	No significant difference in fracture strength was found between $Ca(OH)_2$-filled teeth and untreated control group at different time interval.
Kawamoto *et al*. (Kawamoto et al. 2008)	Bovine	Modulus of elasticity	The sonic velocities of shear and longitudinal waves through known dentine thickness to determine poisson ratio	1, 7, 14, 21, 28, 56 and 90 days	Significant increase in the elastic moduli was detected for dentine specimens exposed to $Ca(OH)_2$ after 7, 14, 21, 28, 56 and 90 days

b) Human studies					
Grigoratos *et al*. (Grigoratos et al. 2001)	Human	Modulus of elasticity Flexure strength	Three-point bend test	1 week	$Ca(OH)_2$ significantly reduced the flexural strength of dentine but not the modulus of elasticity
Yoldaş *et al*. (Yoldas et al. 2004)	Human	Hardness	Knoop microhardness	1, 3 and 7 days	$Ca(OH)_2$ exposure significantly reduced the microhardness after 3 and 7 days
Doyon *et al*. (Doyon et al. 2005)	Human	Fracture resistance	Load to failure	1 and 6 months	USP $Ca(OH)_2$ significantly reduced fracture resistance after 6 months. However, Metapaste did not affect fracture resistance
Rosenberg *et al*. (Rosenberg et al. 2007)	Human	Fracture strength	Microtensile fracture strength	7, 28, and 84 days	$Ca(OH)_2$ significantly reduced microtensile fracture strength after 84 days
Marending *et al*. (Marending et al. 2009)	Human	Modulus of elasticity Flexure strength	Three-point bend test	1, 10, or 30 days	$Ca(OH)_2$ significantly reduced the flexural strength of dentine after 10 days. Modulus of elasticity was not affected
Twati *et al*. (Twati et al. 2009)	Human	Hardness Modulus of elasticity	Nanohardness	1, 3 and 6 months	$Ca(OH)_2$ significantly reduced the dentine nanohardness after 3 and 6 months. Exposure to $Ca(OH)_2$ significantly reduced modulus of elasticity after 6 months
Sahebi *et al*. (Sahebi et al. 2010)	Human	Fracture resistance	Load to failure	1 month	The compressive strength of $Ca(OH)_2$-filled teeth was significantly reduced.

Table 2.8. Summary and main findings of all *in vitro* studies on CH effect on fracture strength included in the review by Yassen and Platt (Yassen & Platt 2013)**.**



Experimental group- teeth dressed with CH; Control group: empty teeth. *above the bars in experimental group and #above the bars in the control group indicate that mean compressive force required to fracture was significantly different from the force required in comparison with the previous time period.

Figure 2.5.The mean compressive force required to fracture the experimental and control group at various time periods (Zarei et al. 2013)**.**

2.5.3. Influence of calcium hydroxide vehicle on fracture resistance

It seems that the CH effect on fracture resistance may be influenced by the mode of CH delivery. Doyon *et al.* (Doyon et al. 2005) showed that there was no difference in fracture resistance of 1-mm human teeth discs exposed to CH solution mixed with saline (USP CH), CH with iodoform (Metapaste) and saline at 30 days. After 180 days, the roots of the teeth exposed to

aqueous CH showed a significant decrease in peak load at fracture when compared to the 30-day groups and the 180-day groups exposed to saline or Metapaste.

2.6. MINERAL TRIOXIDE AGGREGATE

Mineral Trioxide aggregate was developed at Loma Linda University, California and first used as a root-end filling material in endodontic surgery (Torabinejad et al. 1993). When clinical outcomes and scientific observations demonstrated favourable biological responses to the material, other uses of the MTA were explored, including perforation repair, treatment of immature teeth, obturation of teeth with apices wider than 0.7 mm (Buchanan 2001) and pulp capping.

The original MTA was dark grey in colour and was commercialised in 1998; four years later white MTA (WMTA) was introduced.

2.6.1. Chemical properties of MTA

MTA is a mixture of Portland cement and bismuth oxide. It consists of 50-75% (wt) calcium oxide (lime) and 15-25% (wt) silicon dioxide (silica), and bismuth oxide (Bi_2O_3) which is added for radio-opacity. When these raw materials are blended, they produce tricalcium silicate and dicalcium silicate, and small amounts of tricalcium aluminate, tertacalcium aluminoferrite (absent in the white MTA). Other mineral oxides may also be added to improve physical and chemical properties. Calcium sulphate (gypsum) is an important determinant of the setting time.

White MTA consists mostly of tricalcium silicate, and contains significantly lesser amounts of aluminium oxide, magnesium oxide and ferric oxide (Table 2.9) (Asgary et al. 2005).

Elements [a]		Oxides (% wt) [b]				Phases (% wt) [c, d]			
GMTA	WMTA	GMTA		WMTA		GMTA		WMTA	
Ca	Ca	CaO	40.45	CaO	44.26	C_3S		C_3S	
Si	Si	SiO_2	17.00	SiO_2	21.20	C_2S		C_2S	(75)
O	O	Bi_2O_3	15.90	Bi_2O_3	16.13	C_3A	(75)	C_3A	
Fe	Mg	Al_2O_3	4.26	Al_2O_3	1.92	C_4AF			
Mg	Al	MgO	3.10	MgO	1.35				
Al	S	SO_3	0.51	SO_3	0.53	$CaSO_4$ -	(5)	$CaSO_4$ -	(5)
S	Bi	Cl	0.43	Cl	0.43				
Bi		FeO	4.39	FeO	0.40				
		P_2O_5	0.18	P_2O_5	0.21	Bi_2O_3 -	(20)	Bi_2O_3 -	(20)
		TiO_2	0.06	TiO_2	0.11				
		H_2O+CO_2	13.72	H_2O+CO_2	14.49				

Table 2.9. Elemental composition, simple oxides and mineral phases of grey MTA (GMTA) and white MTA (WMTA) (Khan et al. 2014)**.**

2.6.2. Hydration of MTA

Hydration of MTA powder results in a colloidal gel that solidifies into a hard structure. The manufacturer recommends the use of 0.33 g of water with 1 g of ProRoot MTA to achieve an optimum mix of the material.

The hydration of MTA mixed with water has been reported to consist of two separate reactions (Camilleri 2007, Camilleri 2008):

I. The tricalcium silicate and dicalcium silicate react with water to form calcium silicate hydrate and calcium hydroxide:

$$\mathbf{2(3CaO.SiO_2) + 6\ H_2O \rightarrow\ 3CaO.2SiO_2.3H_2O + 3Ca(OH)_2}$$

tricalcium silicate + water → calcium silicate hydrate + calcium hydroxide

$$\mathbf{2(2CaO.SiO_2) + 4H_2O \rightarrow\ 3CaO.2SiO_2.3H_2O + Ca(OH)_2}$$

dicalcium silicate + water → calcium silicate hydrate + calcium hydroxide

II. The tricalcium aluminate reacts with water, and in the presence of calcium sulphate initially produces ettringite:

$$\mathbf{3CaO.Al_2O_3 + CaSO_4 + H_2O \rightarrow\ 3CaO.Al_2O_3.3CaSO_4.31H_2O}$$

tricalcium aluminate +gypsum + water → ettringite

Calcium silicate hydrate and ettringite precipitate on the cement surface, preventing further reactions. The dormant period lasts for 1-2 hours, after that time the hydration of the cement accelerates and more calcium silicate hydrate gel is formed. Hydration of dicalcium silicate also increases at this stage.

When the sulphate-containing phases are depleted, a monosulphate phase is formed from the ettringite. Crystalline calcium hydroxide also precipitates from the liquid phase.

When in contact with tissue fluids and synthetic tissue fluids containing phosphate ions (e.g. Ca- and Mg-free phosphate-buffered saline), the calcium hydroxide produced as a by-product of cement hydration, first reacts to form amorphous calcium phosphate and finally Ca-deficient carbonated apatite (Tay et al. 2007) and is deposited on the cement surface. Carbonated apatite has the general formula:

$(Ca, Mg, Na)_{10}(PO_4HPO_4CO_3)_6(OH)_2$ (LeGeros 1991) and is also known as biologic apatite, and represents the mineral phase of hard tissue (Tay et al. 2007).

A reduction in bismuth oxide was noted after the cement hydration. It has been suggested that bismuth is replacing silicon in the calcium silicate hydrate structure (Camilleri 2008). Bismuth oxide has also been linked to tooth discolouration caused by MTA.

The hydration process can be affected by some restorative materials placed in direct contact with MTA (Camilleri 2011). MTA has the ability to set in the presence of blood, although it may not harden properly, the surface of the MTA showed absence of the acicular crystalline structure formation, and the compressive strength of MTA may be affected adversely as a consequence (Nekoofar et al. 2010, Kim et al. 2012).

2.6.3. Physical properties of MTA

The particle size of MTA is reported as ranging from less than 1 μm to 30 μm, and may be smaller than the diameter of some dentinal tubules (Komabayashi & Spangberg 2008). The authors speculated the smallest particles may enter into open dentinal tubules and provide a hydraulic seal, this may have a role in the superior sealing ability of MTA. In dye leakage investigation comparing white and grey forms of MTA as an apical plug, the grey form of MTA showed significantly less leakage (Matt et al. 2004, Stefopoulos et al. 2008).

The pH value of MTA is 10.2 after mixing and rises to 12.5 at 3 hrs (Torabinejad et al. 1995a). MTA keeps its high pH for an extended period because of the constant release of Ca^{2+} from the MTA and formation of CH (Fridland & Rosado 2003).

The hydration reaction takes several years to complete, although the cement mass will have achieved the final hardening and maximum physical and mechanical properties by 28 days (Camilleri 2015).

The mean hardening time of MTA is 165 ± 5 minutes. The setting time and bacteria leakage are adversely influenced when the samples are kept in dry conditions and keeping MTA in dry conditions decreases its compressive strength (Chogle et al. 2007). Torabinejad and Chivian (Torabinejad & Chivian 1999) recommended placing a wet cotton pellet over MTA; it has

been shown, that placing a moist cotton pellet for the first 24 hours increases the flexularal strength of the material (Walker et al. 2006).

There are conflicting results regarding the MTA setting expansion and its degree of solubility. The water-to-powder ratio might influence the amount of solubility. The higher the ratio, the greater the porosity and solubility (Fridland & Rosado 2003). The authors concluded that using more water would increase Ca^{2+} release from MTA.

The compressive and push-out strength of MTA has been shown to increase significantly and reach their maximum several days after mixing. In one study it was found that values at 3 days were 50 MPa for grey and 45 MPa for white MTA. At 28 days the values were higher: 98 MPa for grey MTA and 86 MPa for white MTA (Islam et al. 2006). This could be explained by the fact that the dicalcium silicate hydration rate is slower than that of tricalcium silicate (Dammaschke et al. 2005).

The microhardness of MTA can be influenced by several factors such as: the pH value of the environment, the thickness and density of the material, humidity, the condensation pressure (Nekoofar et al. 2007), temperature and acid etching (Dammaschke et al. 2005, Nekoofar et al. 2007, Parirokh & Torabinejad 2010).

2.6.4. Influence of pH on MTA properties

The MTA hydration process and both the physical and chemical properties of MTA have been shown to be affected by both low- and high-pH environment.

Effect of acidic pH on MTA

The quality of crystals was assessed as poor in acidic pH conditions compared with an alkaline pH (Lee et al. 2004). MTA had a porous and less crystalline microstructure in the acidic environment, which resulted in reduced microhardness values (Wang et al. 2015).

Bismuth may affect CH precipitation after MTA hydration. Bismuth oxide dissolves in an acidic environment, therefore, it has been suggested that placing MTA in an acidic environment such as inflamed tissues, might result in the release of bismuth oxide and decrease the biocompatibility (Camilleri 2007).

Effect of alkaline pH on MTA

Several studies have demonstrated that MTA may be affected by an alkaline pH during hydration (Hachmeister et al. 2002, Stefopoulos et al. 2008, Saghiri et al. 2009).

Stefopoulos *et al.* (Stefopoulos et al. 2008) examined whether a previous CH intracanal medication affects the sealing ability of MTA and investigated the ability to remove CH from the root canal walls. Four groups of 10 teeth each were created: two groups were treated with CH intracanal medication and then received an apical plug of grey and white MTA respectively. The other groups received an apical plug of grey and white MTA respectively without previous intracanal medication. Four teeth served as negative and one as a positive control. The marginal adaptation and sealing ability of the apical barrier were tested by means of a dye tracer (basic fuchsin) after longitudinal sectioning. It was found that CH pre-treatment adversely affected the sealing ability, leading to the assumption that CH interferes with MTA sealing ability in the apical region. They suggested CH may present a chemical or mechanical obstacle to MTA's adaptation to walls, and chemical interaction might influence the surface characteristics of the MTA.

Saghiri *et al.* (Saghiri et al. 2009) evaluated the morphological microstructure and surface hardness of white MTA after exposure to a range of alkaline environments during hydration. They found that at pH 8.4 and 9.4 the

amount of unhydrated cement decreased and the surface hardness increased. If MTA is exposed to a pH higher than 9.4, adverse effects might result such as more porosity and unhydrated structure. They concluded surface hardness could be influenced by different alkaline pH values (Figure 2.6). However, the results of a bacterial leakage study showed that pretreatment with CH had no significant effect on the sealing ability of MTA as an apical barrier (Hachmeister et al. 2002).

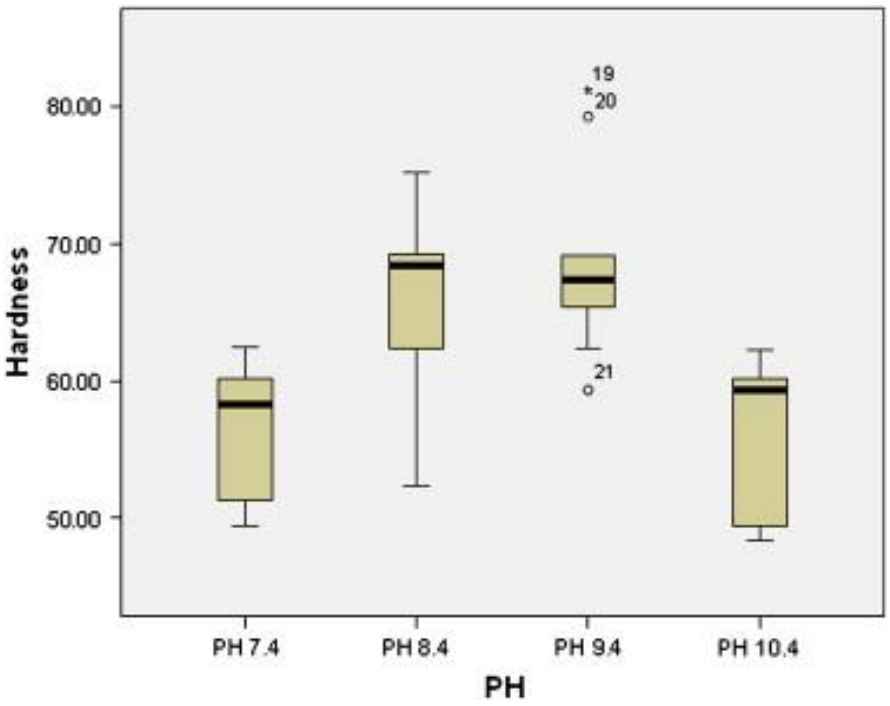


Figure 2.6. Box plots of the surface microhardness of specimens in contact with different pH values.

Illustraction includes the mean ± standard deviation, minimum and maximum amount of microhardness, as well as the variance in each experimental group and outliers (Saghiri et al. 2009).

2.6.5. Influence of MTA thickness and method of placement on MTA

The condensation force may also affect the strength and hardness of MTA. Nekoofar *et al.* (Nekoofar et al. 2007) showed that the surface hardness decreased when more pressure was applied during MTA placement. Greater condensation pressure resulted in fewer voids and microchannels. The authors hypothesized the reduced compressive strength and surface hardness was related to less water uptake that hindered complete MTA setting.

Hand condensation with indirect ultrasonic activation resulted in a denser MTA fill compared with hand condensation alone (Yeung et al. 2006).

A study investigated the bacterial leakage of 1-mm, 2-mm and 3-mm thick MTA apical plugs using a polymerase chain reaction technique. It showed no significant difference between the groups (de Leimburg et al. 2004).

Another study showed that at least a 5 mm apical plug, non-ultrasonically placed, was required to prevent bacterial leakage for 70 days (Al-Kahtani et al. 2005). The addition of indirect ultrasonics during orthograde MTA placement significantly improved the seal of 4 mm (Lawley et al. 2004) and 5 mm (Kim et al. 2009) MTA apical plugs, and was effective at delaying bacterial leakage.

2.7. BIOLOGICAL PROPERTIES OF MTA

2.7.1 Bioactivity of MTA

Physiochemical reactions in MTA and dentine exposed to phosphate containing tissue fluid

Sarkar *et al.* (Sarkar et al. 2005) tested MTA and human teeth filled with MTA stored in a Synthetic Tissue Fluid (STF) for 2 months at 37ºC. The STF was a phosphate buffered saline solution (PBS) (pH=7.2) of the following composition: 1.7 g KH_2PO_4, 11.8 g Na_2HPO_4, 80.0 g NaCl, and 2.0 g KCl in 10L of H_2O.

In part 1 of the study, they measured the concentration of cations that leached from the MTA into STF after 3 days. The concentrations were as follows (mean ± SD, ppm): Ca (calcium) 176.67 ± 3.30, Si (silicate) 13.43 ± 0.58, Bi (bismuth) 6.10 ± 0.45, Fe (ferrite) 2.47 ± 0.40, Al 2.27 ± 0.15, and Mg (magnesium) 1.0 ± 0.1.

They also tested the precipitates collected from the solution after 2 weeks of MTA storage. The precipitates contained mainly O (oxygen), Ca, and P (phosphorous) with trace amounts of Bi, Si, and Al (aluminium).

In part 2 of the study, the researchers examined the MTA-dentine cross-sections using an optical microscope. Their pioneering work discovered an

interfacial layer sandwiched between MTA and the dentinal wall, which appeared to be growing inward and firmly attached to the dentine. The composition of the intermediate layer (Table 2.10) was different from that of MTA (reduced amounts of Al and Si, and presence of P, which is not a component of MTA).

Their data indicated that MTA undergoes dissolution in STF, releasing all of its major cationic constituents. Of all the ions, Ca^{2+} was the most prominent. The authors suggested that because Ca^{2+} is sparingly soluble in biological fluids, it precipitates into hydroxyapatite, according to the following equation:

$$\mathbf{10Ca^{2+} + 6(PO_4)^{3-} + 2(OH)^- \rightarrow Ca_{10}(PO_4)6(OH)_2}$$

The above is a well-known reaction in the biologic calcification process, and is favoured at pH = 7 (LeGeros 1991).

The authors concluded that excellent sealing ability, biocompatibility and dentinogenic activity of MTA result from the physiochemical reactions discussed above.

Bozeman *et al.* (Bozeman et al. 2006) confirmed the findings of Sarkar and associates (Sarkar et al. 2005) by using both grey and white types of MTA. The study showed that both forms of MTA release similar amounts of Ca^{2+} into the PBS. The grey MTA produced more hydroxyapatite crystals compared with WMTA. Tay *et al.* (Tay et al. 2007) showed later that Ca-depleted carbonated apatite was actually formed on the surface.

Area	Ca	Al	Si	Bi	Fe	Mg	O	S	C	P
MTA	21.1	2.6	11.8	7.8	7.5	1.4	41.5	1.3	5.0	-
Interfacial layer	21.5	0.6	3.0	5.6	-	0.1	60.6	-	4.9	3.7
Dentine	31.7	-	-	-	-	0.4	50.8	-	6.0	11.1

Table 2.10. Semi quantitative elemental composition (wt%) of MTA, interfacial layer and dentine (Sarkar et al. 2005).

Bioactivity of MTA

Bioactivity is an expression that describes the beneficial effect of a material implanted in living tissue. The capacity of the material to interact with the living tissue allows the integration of the biomaterial into the environment. *In vitro* bioactivity is measured by the ability to produce carbonated apatite in the presence of phosphate-containing fluid (Tay et al. 2007).

Reyes-Carmona *et al*. (Reyes-Carmona et al. 2009, Reyes-Carmona et al. 2010a, Reyes-Carmona et al. 2010b) and Dreger *et al.* (Dreger et al. 2012) in a series of articles studied the interfacial layer formed between MTA and dentine.

Their work confirmed the findings of Sarkar *et al.* (Sarkar et al. 2005). They suggested that the interfacial layer, with tag-like structures entering the dentinal tubules was formed as a result of biomineralization (Figure 2.7). Ca^{2+} ions released from the MTA diffused through the dentinal tubules and reacted with Ca-free and Mg-free PBS, also known as STF, and produced calcium phosphate. Calcium phosphate incorporated other ions and matured into carbonated apatite (Figure 2.8).

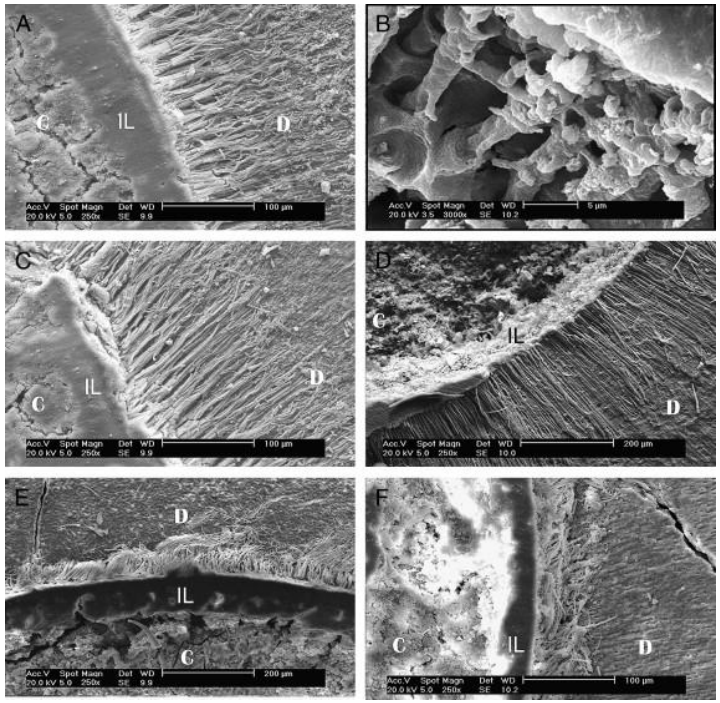
Han *et al.* (Han et al. 2010) undertaken morphological and chemical analysis of precipitates of MTA stored in either distilled water or PBS after 1 and 14 days. They discovered that calcium carbonate and calcium hydroxide were formed on the surface of MTA when stored in distilled water, whereas

amorphous calcium phosphate crystals were formed on the surface of cement immersed in PBS.

It has also been shown that when MTA was sealed with a wet cotton pellet for 72 hours it produced superficial precipitation, occluding or partially occluding dentinal tubules, with elemental composition similar to MTA. When MTA was stored for 2 months in Ca- and Mg-free PBS, tag-like structures containing mainly Ca and P, were found deeper in dentinal tubules (Reyes-Carmona et al. 2010a). This phenomenon suggested the possibility that the precipitated minerals formed the mineralised interfacial layer which produced chemical bonding between MTA and dentine, and that this can be responsible for the superior sealing ability (biological seal) of MTA, prevent marginal leakage (Martin et al. 2007) and MTA root filling displacement (Reyes-Carmona et al. 2010a).

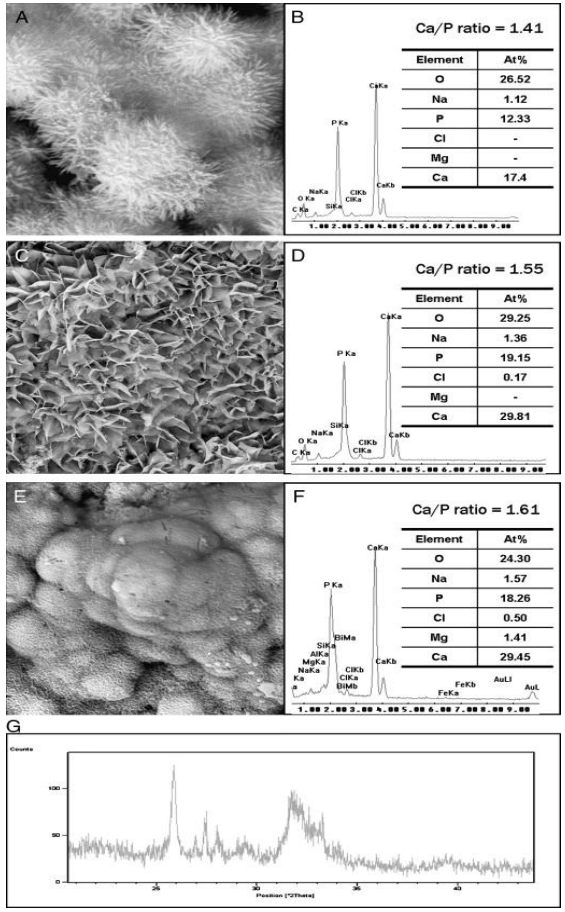
The formation of carbonated apatite on the material surface has been reported to be the reason for the bioactivity of MTA (Sarkar et al. 2005, Bozeman et al. 2006, Tay et al. 2007, Reyes-Carmona et al. 2009).

Hardened MTA, on exposure to moisture was shown to release calcium and hydroxyl ions, which could be responsible for its cementogenesis-inducing properties (Santos et al. 2005, Fridland & Rosado 2005, Bozeman et al. 2006, Shie et al. 2009).



A) Photomicrograph of ProRoot MTA–dentin interface showing cement (C), interlayer (IL), and dentin (D). (B) Higher magnification showing TS and lateral branches. Photomicrograph of (C) MTA Branco–dentin interface, (D) MTA BIO–dentin interface, (E) PC1-dentin interface, and (F) PC2-dentin interface. (*E*) and (*F*) show the formation of fewer, short TS.

Figure 2.7. Photomicrographs showing that the interfacial layer, with tag-like structures entering the dentinal tubules (Reyes-Carmona et al. 2009)**.**



A) High-magnification SEM showing the acicular nature of spherules (original magnification, 8000×). (*B*) EDAX spectrum for precipitates in (*A*) and semiquantitative chemical composition showing their Ca/P molar ratio. (*C*) Photomicrograph showing petal-like precipitates (original magnification, 1000×) for which (*D*) SEM-EDAX revealed a greater Ca/P molar ratio and lattice substitution of Na and Cl. (*E*) SEM of compact lath-like precipitates (original magnification, 1000×) that demonstrates (*F*) a Ca/P molar ratio of 1.61 with lattice substitution of Na, Cl, and Mg. (*G*) X-ray diffraction pattern of the calcium phosphate precipitate obtained after 2 months of immersion in PBS, revealing the presence of a weakly crystalline apatite.

Figure 2.8. Morphologic characterization of precipitates formed by MTABIO after 2-month immersion in phosphate-buffered saline (Reyes-Carmona et al. 2009)**.**

The antibacterial and antifungal properties of MTA have been extensively evaluated, with conflicting reports (Parirokh & Torabinejad 2010). Because of its alkaline pH, MTA was shown to have an antibacterial effect on some facultative bacteria and no effect on strict anaerobes (Torabinejad et al. 1995a). The concentration of MTA may be a significant factor in the antifungal effect of MTA (Al-Hezaimi et al. 2005).

In summary, MTA acts as a bioactive material when placed in direct contact with human tissues, and has the following properties:

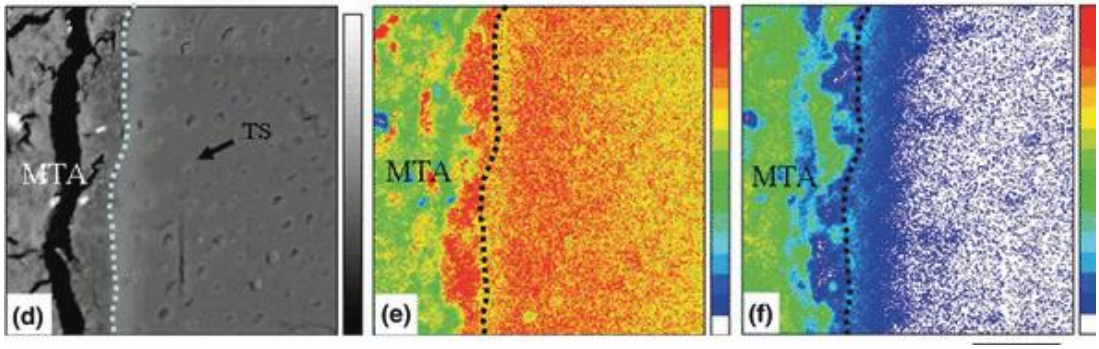
- Forms CH and releases Ca^{2+} ions for cell attachment and proliferation (Sarkar et al. 2005, Fridland & Rosado 2005, Bozeman et al. 2006, Tay et al. 2007, Camilleri 2008, Shie et al. 2009),
- Forms carbonated apatite on its surface and provides a biologic seal (Sarkar et al. 2005, Bozeman et al. 2006, Reyes-Carmona et al. 2009, Dreger et al. 2012),
- Its alkaline pH creates an antibacterial environment (Torabinejad et al. 1995a, Fridland & Rosado 2005, Parirokh & Torabinejad 2010),
- Encourages differentiation and migration of hard tissue-producing cells (Shabahang et al. 1999, Kuratate et al. 2008),
- Modulates cytokine formation (Huang et al. 2005).

2.7.2. Element diffusion from MTA into dentine

Dentine may uptake several elements released from bioactive materials, such a phenomenon may cause chemical, mechanical and structural modification of dentine (Hotta et al. 2001). The element incorporation by adjacent dentine may be regarded as an indicator of the material's bioactivity.

Han and Okiji (Han & Okiji 2011) investigated the uptake of Ca and Si from white MTA by bovine dentine in the presence of Ca- and Mg-free PBS. The teeth were copiously irrigated with 3% NaOCl during preparation. Final irrigation with a chelating agent and NaOCl was carried out before MTA placement. The samples were stored for 1, 7, 30 or 90 days in Ca- and Mg-free PBS.

Chemical component bulk analysis and element mapping for the 60-70 μm dentine adjacent to MTA was carried out using SEM-Electron Probe Micro-analyser (EPMA). The examination revealed Ca and Si-rich dentine areas along the MTA-dentine interface (Figure 2.9).



WMTA–dentine interfacial areas of representative specimens after 30-day PBS immersion. d) SEM image, e) Ca mapping, f) Si mapping, TS= tag-like structure. Dotted lines show the approximate interface position (bar, 20 μm; grey and colour bars, X-ray strength).

Figure 2.9. SEM micrographs (d) and mapping images for Ca (e) and Si (f) obtained by SEM-EPMA (Han & Okiji 2011)**.**

The composition of the interfacial dentine layer adjacent to MTA is presented in Table 2.11. The results of the study demonstrated that dentine in contact with MTA incorporated Ca and Si from the MTA. The elements seemed to penetrate deeper into dentine, the longer the teeth were stored in PBS (Table 2.12). The authors suggested that the mineral uptake caused chemical and structural changes in dentine, which might result in higher acid resistance and increased physical strength of the dentine.

Elements	Control	PBS immersion for			
		1 day	7 days	30 days	90 days
Ca	32.3 (0.3)	36.3 (6.0)	37.1 (6.2)	37.9 (6.4)	40.2 (5.6)
O	26.8 (0.3)	27.6 (5.9)	28.8 (4.1)	25.2 (1.7)	24.7 (5.0)
P	26.5 (1.0)	23.0 (3.3)	22.6 (3.2)	23.1 (4.4)	23.1 (3.5)
C	12.1 (1.0)	8.9 (0.4)	7.1 (1.0)	9.8 (1.4)	7.5 (1.1)
Si	0.0	1.0 (0.2)	0.8 (0.1)	1.0 (0.7)	1.2 (0.2)
Ca/P	1.2 (0.1)a	1.6 (0.3)ab	1.7 (0.2)b	1.7 (0.2)b	1.8 (0.2)b

Mean (SD), n = 5. Mean values followed by different letters (Ca/P) are significantly different ($P < 0.05$).

Table 2.11. Principal composition of the interfacial dentine layer adjacent to MTA (atomic %) (Han & Okiji 2011)**.**

PBS immersion	Ca	Si
1 day	14.4 (3.8)	13.8 (2.2)
7 days	77.8 (13.5)	61.0 (8.9)
30 days	166.6 (10.1)	115.4 (24.0)
90 days	206.6 (15.1)	171.2 (33.4)

Mean (SD), n = 5.

Table 2.12. The incorporation depths of calcium and silicon into dentine (μm) (Han & Okiji 2011)**.**

2.7.2. Biocompatibility of MTA

Several tests *in vitro* and *in vivo* have been used to examine the biocompatibility of MTA, including:

- Mutogenicity (Kettering & Torabinejad 1995),
- Neurotoxicity and neurologic effects (Asrari & Lobner 2003, Abbasipour et al. 2009),
- Vascular effect (Masuda et al. 2005),

- Cell cultures (Torabinejad et al. 1995b, Zhu et al. 2000, Perez et al. 2003, Camilleri et al. 2004, Kuratate et al. 2008).

The results of a meta-analysis on MTA biocompatibility when used as a root end filling in endodontic surgery showed that MTA was more compatible than reinforced zinc oxide-eugenol cements: Super ethoxy benzoic acid (EBA) and IRM as well as amalgam (Fernandez-Yanez Sanchez et al. 2008).

2.8. CALCIUM HYDROXIDE VS MTA APEXIFICATION

2.8.1 Treatment outcome studies

In an *in vivo* study in dog model, a three month treatment with CH or MTA resulted in comparable amounts of hard tissue formation, the MTA group showed significantly greater consistency and predictability (Shabahang et al. 1999).

In another *in vivo* study, human teeth either underwent MTA apexification after 7 days of disinfection with CH, or CH apexification and obturation which was performed following clinical and radiographic determination of the apical stop. The mean time taken for apical biological barrier formation was 3 +/- 2.9 months for teeth obturated with MTA and 7 +/- 2.5 months for teeth that underwent CH apexification The time taken to complete the treatment and the biological barrier formation in the MTA group was significantly less than that for CH group ($P = 0.008$) (Pradhan et al. 2006).

A systematic review and meta-analysis comparing apexification with CH and MTA based on clinical outcome and apical barrier formation was published in 2011. Based on two studies, no difference between those treatment modalities was reported (Chala et al. 2011).

The studies included were:

- El-Meligy and Avery (El-Meligy & Avery 2006) in their split mouth study comparing MTA and CH apexification techniques, showed 100% clinical and radiographic success of MTA apexification after 12 months. They concluded, MTA was a suitable replacement for CH for the apexification procedure.

- Pradhan *et al.* (Pradhan et al. 2006) have shown the healing time for periapical radiolucencies after MTA and CH apexification was almost identical, but the time taken to complete the treatment and the biological barrier formation in the MTA group was significantly less than that for CH group.

2.8.2. Fracture resistance studies

Fracture resistance of teeth obturated with MTA

Hatibović-Kofman *et al.* (Hatibovic-Kofman et al. 2008) tested the fracture resistance of immature sheep roots restored with MTA, dressed with CH and untreated teeth, at various time intervals from 2 weeks to 1 year and stored at 4^{o}C. In their study the untreated teeth showed the highest value at 2 weeks, and after 2 months the fracture strengths decreased significantly. There was no difference in the fracture resistance between the groups at 2

weeks and 2 months. However, the strength was significantly higher for the MTA-treated group compared with the other two groups after 1 year. It was hypothesized that MTA induced the expression of a tissue inhibitor of metalloproteinase-2 in the dentine matrix, and thus possibly prevented destruction of the collagen matrix.

Tuna *et al.* (Tuna et al. 2011) investigated the fracture resistance of human immature premolar teeth. The teeth received treatment by apical approach and were filled with MTA or CH, and stored in saline at 4ºC for 1 year. No control group was used in this study. The CH group showed lower resistance to fracture than the MTA group.

Milani *et al.* (Milani et al. 2012) investigated fracture resistance of human teeth prepared to resemble immature teeth, with apices of size 170 ISO. The teeth received irrigation with 5.25% NaOCl, were obturated with MTA and stored for 6 months in a sponge moistened with PBS. The MTA-filled teeth showed higher fracture resistance in comparison to untreated teeth ($P < 0.05$).

In another study (EL-Ma'aita et al. 2014) freshly extracted human teeth were decoronated, prepared and filled with either MTA, gutta-percha and sealer or unfilled and stored for 48 hr, 1, or 6 months at 37ºC in PBS. Following the storage periods, the roots were mounted in acrylic supports, and the periodontal ligament was simulated using an elastomeric impression material. The samples underwent fracture resistance test. The results are presented in Figure 2.10. Two modes of fracture were indentified: split and comminuted. They concluded that MTA increased the resistance to vertical

root fracture of endodontically treated teeth and influenced the mode of fracture after 1 and 6 months of storage in PBS as compared to gutta-percha and sealer- treated teeth.

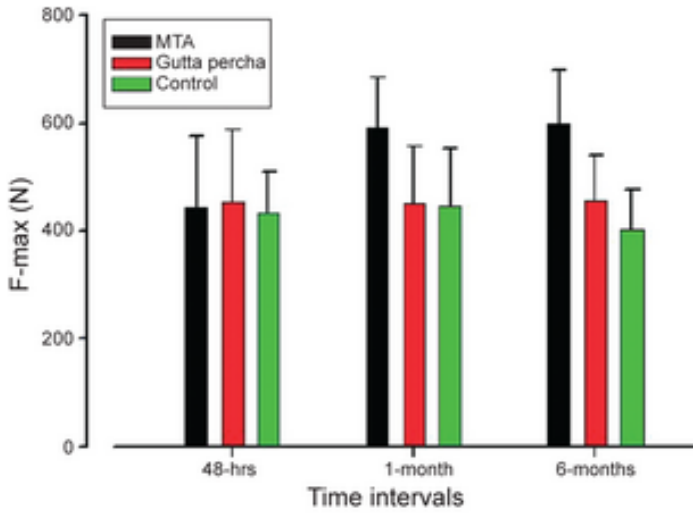


Figure 2.10. Bar chart illustrating the means and standard deviations of the maximum force at fracture within each group (EL-Ma'aita et al. 2014)**.**

Bayram & Bayram (Bayram & Bayram 2016) also investigated fracture resistance of human teeth that were prepared to resemble immature teeth, with apices of size 170 ISO. Samples that received no treatment and those obturated with MTA were stored at 37ºC and 100% relative humidity for 7 days. The study also found that the teeth obturated with MTA had higher resistance than the control.

Fracture resistance of teeth pre-treated with CH and obturated with MTA

Andreasen *et al.* (Andreasen et al. 2006) examined the fracture resistance of immature sheep kept in saline for 100 days at 6ºC. Four root groups were tested: dressed with CH, restored with MTA, premedicated with CH for 30 days before MTA placement and control, empty roots. They found that teeth dressed with CH were significantly less resistant to fracture than control and MTA groups. They found no difference in fracture resistance between control, MTA and MTA that received pre-medication with CH groups.

pH changes in dentine

In an *in vitro* study using matched pairs of human teeth, the effects of intracanal MTA and CH on hydroxyl ion diffusion through dentine was assessed by measuring pH changes over time. The teeth were filled with either white MTA or CH, control teeth were filled with saline. The pH in root surface cavities was measured at 3 hours, 24 hours, 1 week, 2 weeks, 3 weeks, and 4 week. The overall mean pH was found to be higher in the MTA group (8.66 ± 0.07) compared with calcium hydroxide (8.46 ± 0.07), and this difference was significant. At 4 weeks, the pH in MTA group (8.3) was still significantly higher than CH (7.9), but not at any other time interval (Heward & Sedgley 2011).

Another study investigated pH changes in teeth filled either with white MTA or CH, on simulated resorption cavities prepared at 5 mm and 2 mm from the apex. The teeth were sealed coronally and apically and immersed in saline. The pH in root surface cavities was measured at 20 minutes, 3 hours, 24 hours, 1 week, 2 weeks, 3 weeks, and 4 weeks. The pH at 5 mm when compared with the 2-mm level was significantly higher for both the MTA and CH. At both the 2-mm and 5-mm levels, significant pH changes occurred over time in the MTA, and CH. The authors concluded that the pH was time and root level dependant (Hansen et al. 2011).

2.9. RELEVANCE TO THE CURRENT PROJECT

This chapter has outlined the materials and techniques used for apexification of immature teeth and their influence on dentine properties.

There has been no study comparing fracture resistance of human teeth that addressed the following issues simultaneously:

- Current irrigation protocols during root canal preparation.
- Teeth that had received treatment with CH, MTA and CH followed with MTA.
- Teeth stored (at 37º in the presence of PBS) and tested (fracture tests with simulated periodontal ligament, load applied at 130º to the long axis of the tooth in a lingual-labial direction) in simulated clinical conditions.

The physiochemical reactions in MTA and dentine exposed to phosphate-containing tissue fluid have been already described thoroughly in the literature, but so far no study has investigated the properties of MTA mixed with Ca- and Mg-free PBS and evaluated its effects on dentine properties, the possibility of primary monoblock formation between the cement and dentine, its effect on fracture resistance, as well as the influence of CH on MTA mixed with PBS.

2.10. CHAPTER TWO CONCLUSIONS

The primary motivator for researching MTA apexification and its influence on the fracture resistance of human teeth is the impact this could have for the retention of root canal treated teeth.

MTA mixed with water has been shown to have excellent sealing ability, biocompatibility and hard tissue-inductive activity when the hydration of the material process takes place in the presence of phosphate-containing fluid at pH around 7. Notwithstanding this the effects of a change in the pH of human dentine, caused by CH pre-treatment before MTA apexification, on the fracture resistance of human teeth has not been investigated. There is also no study investigating the effect of MTA mixed with PBS on the fracture resistance of such teeth and its potential to form a primary monoblock with radicular dentine.

As the presence of calcium hydroxide may weaken human teeth and the presence of MTA may bring about hard tissue-inductive activity countering this, such effects are worthy of investigation.

This project will attempt to establish the most appropriate clinical protocol for treatment of teeth with wide apices. The importance of the possible weakening effect of the long-term use of CH may have on human teeth, and its unknown effect on the biological properties of MTA justifies the current project.

CHAPTER THREE: AIMS AND HYPOTHESES

3.1. AIMS OF THE STUDY

The aim of this study is to test, *in vitro*, the fracture strength of extracted human teeth with apical plugs of Mineral Trioxide Aggregate mixed with either water or Ca- and Mg-free Phosphate Buffered Saline, and to establish whether these materials strengthen the root. An analysis will be made of the interface between the material and the tooth structure using a Scanning Electron Microscope (SEM) equipped with an Energy Dispersive X-ray detector (EDX) to observe potential mineral exchange at this interface.

Research questions

1. Does the placement of MTA in the apical 5 mm of the root canal strengthen the root of immature permanent human teeth by bonding chemically to the tooth structure?
2. Does the placement of a disinfecting dressing of calcium hydroxide have an adverse effect on the fracture resistance of human teeth filled with MTA apical plugs?
3. Does an apexification with MTA mixed with Ca- and Mg-free Phosphate Buffered Saline have the same effect on the fracture resistance of immature human teeth as MTA mixed with water?

3.2. NULL HYPOTHESES TESTED BY THIS WORK

1. Placement of MTA apical plug has no influence on the vertical root fracture resistance of immature human teeth.

2. Pre-treatment with a disinfecting dressing of calcium hydroxide does not have an adverse effect on the fracture resistance of immature human teeth filled with MTA apical plugs.

3. An apexification with MTA mixed with Ca- and Mg-free PBS has the same effect on fracture resistance of immature human teeth as apexification with MTA mixed with water.

CHAPTER FOUR: MATERIALS AND METHODS

4.1. SAMPLE TEETH

4.1.1. Teeth collection

One hundred and eighty extracted single-rooted human teeth were used in this study. The collection of teeth was approved by the East of Scotland Research Ethics Service (Appendix I) and the Tayside Medical Science Centre (Appendix II). The teeth were extracted as part of a consented treatment plan, from patients who required tooth removal for some other reason, mostly for orthodontic reasons or because of advanced periodontal disease. Verbal and written (Appendices III and IV) consent was taken for each patient. Teeth were stored in sterile water at room temperature, in a locked cabinet in Dundee Dental Hospital and School.

The experimental work in this section used a wide variety of materials, instruments and equipment. The details of these are summarised in Table 4.1.

Name	Manufacturer
ApexCal®	Ivoclar Vivadent, Schaan, Liechtenstein
ApexCal® syringe applicator	Ivoclar Vivadent, Schaan, Liechtenstein
B&L Condenser, size 60/120	B&L Biotech Inc., Fairfax, VA, USA
B&L Ultrasonic endodontic tip	B&L Biotech Inc., Fairfax, VA, USA
Chloraxid® 5.25%	PPH Cerkamed, Stalowa Wola, Poland
Citric acid	Dundee Dental Hospital laboratory, Dundee, Scotland
Classic Etch Gel	37% phosphoric acid gel
Coltosol® R	Coltène Whaledent AG, Altstätten, Switzerland
Cotton wool rolls	Wright Health Group Ltd., Dundee, Scotland
Dental operating microscope	Carl Zeiss Microscopy, Zeiss, Germany
Excavator	UnoDent Ltd., Manchester, UK
Impregum™ F	3M ESPE, Seefeld, Germany
INCAx-sight EDX detector	Oxford Instruments, Abingdon, UK
Instron® Testing Machine 4449	Instron, High Wycombe, UK
Iwanson dental gauge caliper	Tools N Tools, Birmingham, UK
JEOL JSM-5600 Scanning Electron Microscope	JEOL USA Inc., Peabody, MA, USA

Name	Manufacturer
Largo Peeso reamers, sizes 1-5	Dentsply Maillefer, Ballaigues, Switzerland
Modelling Wax	Associated Dental Products Ltd., Swindon, England
Monoject™ Endodontic syringe 3CC 23G	Covidien, Greenwood, SC, USA
MTA disposable carrier, size 1.6 mm	Vista Dental Products, Racine, WA, USA
NiTiFlex® k-file, sizes 8-40 ISO	Dentsply Maillefer, Ballaigues, Switzerland
Orthoresin	Dentsply DeTrey, Hanau-Wolfgang, Germany
Paper points, size 140 ISO	UnoDent Ltd., Manchester, UK
ProRoot™ MTA white	Dentsply, Tulsa, OK, USA
ProTaper® Universal rotary files, sizes SX-F5	Dentsply Maillefer, Ballaigues, Switzerland
Satelec P5 Newtron	Acteon, Saint Neots, UK
Skilldenta diamond disc	Skillbond Direct Ltd., High Wycombe, UK
Surveyor	Mestra® Engineering Ltd. Helsinki, Finland
Thermostatically controlled incubator model M30C	Genlab, Widens, England
UnoDent FG Diamond 745 Coarse	UnoDent Ltd., Manchester, UK

Table 4.1. Summary of materials, instruments and equipment used in the study, and the manufacturers' details.

4.1.2. Sample selection

The teeth were examined under magnification for root caries, cracks, fractures and root resorption. Only teeth without this were selected for use in the study. Thereafter the crown of each suitable tooth was removed by separating it from its root with a disc (Skilldenta) at the level of the labial cemento-enamel junction. The apical portion of the root was also removed in the same way parallel to the cut coronal root surface, leaving a 10 mm long root. Following this the root canal was instrumented with ISO size 08-15 k-files, and the dental pulp was removed. The teeth were examined again under a dental operating microscope at 16x magnification to exclude any cracks or fractures arising from this procedure. Teeth with cracks or fractures, sclerosed canals, more than one root canal, or resorption were excluded from the study.

4.1.3. Sample preparation

The root canals of the selected teeth were enlarged to create thin-walled samples, resembling immature roots. In order to achieve this the root canal space was prepared using nickel-titanium k-files (NiTiFlex®, sizes 08-40 ISO), with no lubricant, and rotary nickel-titanium files (ProTaper® Universal) up to a size F5. Largo Peeso drills, sizes 1-5, with a size 5 drill (size 150 ISO) introduced 1 mm through the apical root-end were used to complete root canal enlargement (Figure 4.1A). During the preparation of each tooth a standardised 6 ml of 5.25% NaOcl (Chloraxid®) irrigant was used. After

preparation the roots were irrigated with 10% citric acid (CA) and 3 ml of NaOCl and dried with paper points. The teeth were stored in sterile water at room temperature until the start of the experiment.

The remaining root wall thickness was measured with a dental crown caliper at 1 mm, 3 mm and 5 mm from the apical end. The roots were then divided into nine groups, with twenty roots in each group, according to the thickness of the thinnest root wall, as detailed in Table 4.2. This distribution of teeth accordingly to wall thickness was the same for all experimental and control groups.

Number of teeth in each group	**Minimal root thickness**	**Number of walls affected**
1	0	1-2 walls
2	0	1wall
1	≤ 0.2 mm	1 wall
3	< 0.5 mm	2 walls
3	< 0.5 mm	1 wall
2	0.5 mm-0.8 mm	1 wall
4	0.8 mm-1.0 mm	1 wall
2	1.0 mm-1.2 mm	1 wall
1	1.2 mm-1.5 mm	1 wall
1	≥ 1.5 mm	1 wall

Table 4.2. Number of roots with a specific minimal wall thickness in each group.

4.1.4. Allocation to experimental and control groups

Prepared and grouped roots were randomly allocated to six experimental groups, and three control groups as detailed in Tables 4.3 and 4.4.

Group name	Treatment received
2/52 CH +MTA(W)	2 weeks dressing with calcium hydroxide, irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite, 5 mm MTA (mixed with sterile water) apical plug
12/52 CH + MTA(W)	12 weeks dressing with calcium hydroxide, irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite, 5 mm MTA (mixed with sterile water) apical plug
MTA(W)	Irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite, 5 mm MTA (mixed with sterile water) apical plug
2/52 CH +MTA(PBS)	2 weeks dressing with calcium hydroxide, irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite, 5 mm MTA (mixed with Ca- and Mg-free phosphate buffered saline) apical plug
12/52 CH + MTA(PBS)	12 weeks dressing with calcium hydroxide, irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite, 5 mm MTA (mixed with Ca- and Mg-free phosphate buffered saline) apical plug
MTA(PBS)	Irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite, 5 mm MTA (mixed with Ca- and Mg-free phosphate buffered saline) apical plug

CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS)= MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 =dressed for 12 weeks.

Table 4.3. The experimental groups and summary of treatment they received.

Group name	Treatment received
IRRIGATION ONLY	Irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite
2/52 CH	2 weeks dressing with calcium hydroxide, irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite
12/52 CH	12 weeks dressing with calcium hydroxide, irrigation with 3 ml of 10% citric acid and 3 ml of 5.25% sodium hypochlorite

CH = Calcium hydroxide, 2/52 = dressed for two weeks, 12/52 =dressed for 12 weeks.

Table 4.4. The control groups and summary of treatment they received.

4.2. TREATMENT PROCEDURES

4.2.1. Groups with calcium hydroxide dressing

The root canal was dried and filled with a polyethylene glycol-based CH paste (ApexCal®) using a syringe applicator. The coronal portion of the root canal was sealed with a 2 mm-thick zinc oxide/zinc sulphate-based dressing (Coltosol® R). Depending of the group allocation, the samples were stored for either 2 or 12 weeks. After that time, the coronal dressing was removed, the teeth were irrigated with 3 ml of CA and 3 ml of NaOCl and dried with paper points.

4.2.2. Groups with MTA

White MTA (ProRoot™ MTA) was mixed either with sterile water or calcium-free and magnesium-free phosphate-buffered saline, using 0.33 g of liquid and 1 g of MTA. The PBS containing 136.4 mM NaCl, 2.7 mM KCl, 8.2 mM NaH_2PO_4 and 1.25 mM KH_2PO_4 in deionized water (pH 7.2). This formulation was described previously by Reyes-Carmona *et al.* (Reyes-Carmona et al. 2009).

In groups with CH pre-medication, no further irrigation was used. In other groups, the samples were irrigated with 3 ml of CA and 3 ml of NaOCl, to ensure the same total quantity of irrigation solutions were used for all samples. This was introduced into the canal system using an endodontic

syringe (Monoject™). Once completed the root canals were dried with paper points before MTA placement.

White MTA was used to obturate the apical 5 mm of the roots (Figure 4.1B). Increments of cement were transferred into the root canal using a disposable MTA carrier, and condensed manually using a plugger (B&L Condenser). During MTA condensation ultrasonic energy (Satelec P5 Newtron and B&L Ultrasonic endodontic tip) was applied to the plugger for 5 seconds. A moist cotton pellet was placed over MTA (cotton pellets were soaked in either sterile water or PBS, to match the liquid used to mix MTA), and sealed with temporary cement (Coltosol® R). The samples were stored for 4 weeks.

4.2.3. Irrigation only group

The samples allocated to this group were initially prepared according to paragraph 4.2.2.

To complete the preparation of the samples in this group the samples were further irrigated with 3 ml of CA and 3 ml of NaOCl and dried with paper points (Figure 4.1A). The coronal part of the root was sealed with a 2 mm layer of the temporary dressing (Coltosol® R). The prepared roots were stored for 4 weeks.

4.2.4. Storage

All samples were stored for at 37ºC and in 100% relative humidity in a thermostatically controlled oven. Relative humidity of the specimens was maintained at 100% by keeping the samples in closed containers with 4 cotton wool rolls soaked in Ca-free and Mg-free PBS.

4.3. FRACTURE RESISTANCE TESTING

4.3.1. Preparation of samples for fracture test

The temporary dressing was removed from the coronal portion of the roots of each specimen. To facilitate specimen mounting a line was drawn around the circumference of each sample using a pencil, 2 mm from the coronal end of the root. Another line was drawn on the coronal surface of the root, 2 mm from the palatal / lingual margin (Figure 4.1C). A 2-mm deep grove was cut on the palatal / lingual aspect of the coronal root-end using a diamond fissure bur (UnoDent FG No.745) in a water-cooled high speed handpiece (Figure 4.1D).

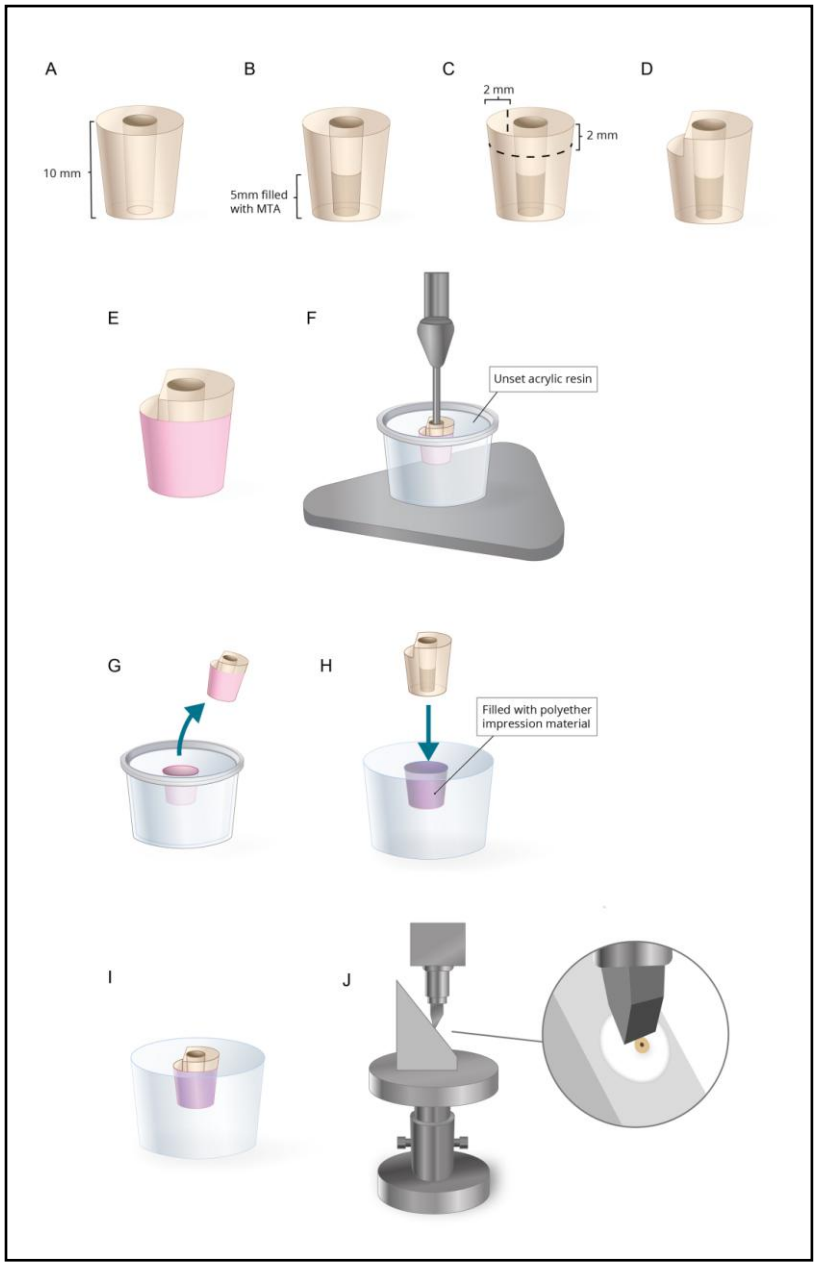


Figure 4.1. Preparation of samples for fracture resistance test.
A – Sample with no MTA filling, B - Sample filled with MTA apical plug, C - Orientation lines for cutting a grove and mounting the samples in acrylic moulds, D - A groove created at the palatal / lingual aspect of each sample to facilitate placement of the tip of the chisel of the Instron® machine, E – sample dipped in molten wax, F - Sample submerged into plastic cylindrical mould filled with freshly mixed self-curing acrylic resin to the depth indicated by the marked line, a surveyor was used to ensure perpendicular sample placement in the resin, G - Once the setting of the acrylic resin was complete, the sample was removed from the resin. Residual wax from the sample surface and the the base of the created “socket” was removed, H - Sample was re-inserted into the “socket” filled with polyether impression material, and the excess impression material removed, I - Sample ready for fracture test after the material was allowed to fully set, J - Sample set up in the Instron® machine using a metal jig.

4.3.2. Embedding of samples and periodontal ligament simulation

18 prepared roots from each sample group were randomly selected to undergo fracture resistance tests. In order to prepare these for further testing the roots were dipped in molten wax for 1s up to the line marked 2 mm from the coronal root surface (Figure 4.1E). This resulted in deposition of a 0.2 - 0.3 mm layer of wax. Thereafter the roots were submerged into plastic cylindrical moulds filled with freshly mixed self-curing acrylic resin to the depth indicated by the marked line. In the resultant specimen a 2-mm gap between the cement-enamel junction (CEJ) and the top of the resin simulated the anatomical spacing found between the bone and the CEJ, as suggested by Wilkinson *et al.* (Wilkinson et al. 2007). In this process a surveyor was used to ensure perpendicular sample placement in the resin (Figures 4.1F and 4.2).

Once the setting of the acrylic resin was complete, the samples were removed from the resin (Figure 4.1G). Residual wax at the base of the created “socket” was removed from the acrylic mount using boiling water followed by drying by application of air from a dental triple syringe. Wax from the root ends was scraped off with a sharp spoon excavator, and any further residue was removed by rubbing the specimen end with gauze after dipping the root in boiling water for 1s to soften the wax. Acrylic cylinders were removed from the plastic moulds and their “sockets” were filled with polyether impression material (Impregum™), as previously recommended as a material of choice to simulate the periodontal ligament (Soares et al. 2005).

a) b)

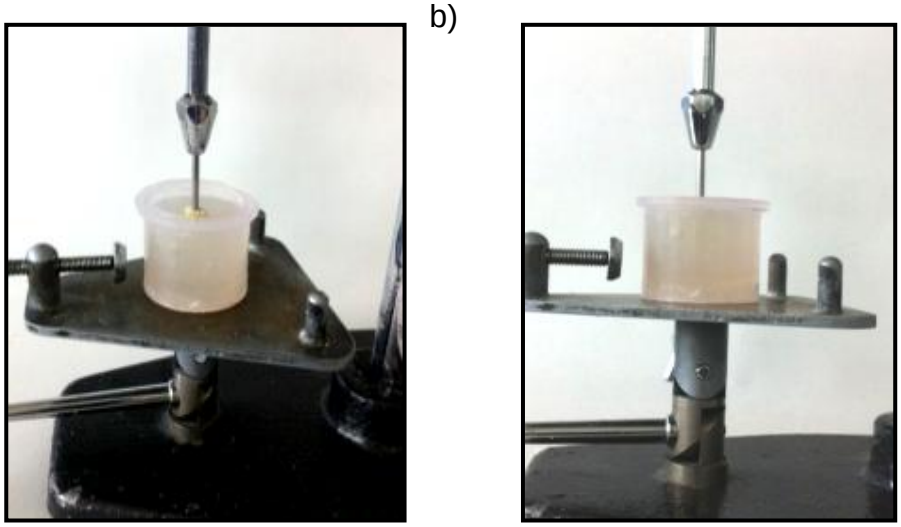


Figure 4.2 a, b. Images demonstrate sample embedding in the orthodontic resin using a surveyor.

Prior to the impression material setting the roots were re-inserted into the “socket”, and the excess impression material removed (Figure 4.1H). The material was then allowed to set fully (Figure 4.1I). The fracture resistance tests were undertaken the following day prior to which the completed specimens were stored dry at room temperature.

A variant of this procedure applied to the root canals of teeth with no MTA apical plug where paper points were placed in the root canals prior to roots being dipped in wax, placed in the orthodontic resin and re-inserted into the “socket” filled with polyether impression material. This thus ensured that no material entered the root canal.

4.3.3. Fracture resistance test

To carry out this work a metal jig was designed and fabricated for the purpose of this study. This permitted the prepared tooth specimens to be loaded by the tip of a chisel to destruction at 130º to the long axis of the tooth in a lingual-labial direction, using an Instron® Universal testing machine (Figure 4.3a) at a crosshead speed of 5 mm / min. This experimental set up is shown in detail in Figures 4.1J and 4.3b.

All specimens were tested to fracture and the maximum force at fracture (F-max) was recorded in Newtons (N). The roots were then removed from the acrylic “sockets” and inspected for vertical root fractures, the fracture depth and mode were recorded for each sample, using a structured data collection sheet as presented in Table 4.5. This permitted the recording of two modes of fracture: split or comminuted. An oblique fracture split a root into two parts, whereas comminuted fracture had multiple planes and resulted in the root being broken into several parts. Three depths of fracture: at the level of the cylinder, into the cylinder and vertical root fracture were recorded.

a)

b)

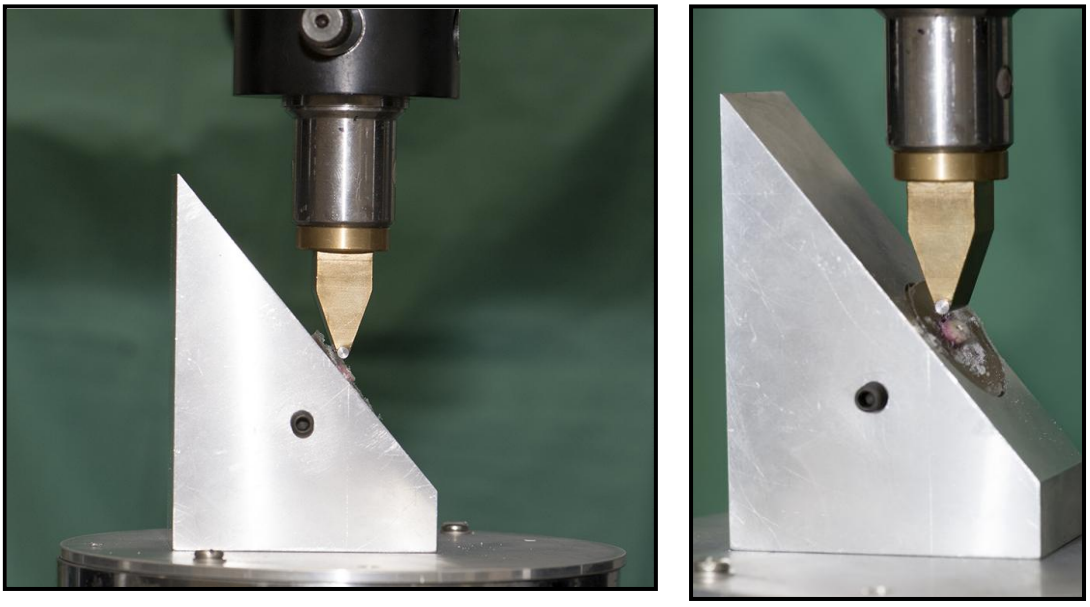


Figure 4.3. Sample mounting in the Instron® Testing Machine.
a) Instron® Testing Machine, b) Specimens fixed in a jig, with a chisel-shaped tip applied at 130º to the long axis of the tooth in a lingual-labial direction.

Sample No.	F-max [N]	Fracture type		Depth of fracture		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1						
2						
3						
4						

Table 4.5. Structured data collection table used to record the fracture resistance results.

4.4. ELEMENT ANALYSIS

4.4.1. Sample preparation

Two teeth per group, which had not undergone the fracture resistance testing following preparation were used for the analysis. The teeth were sliced perpendicular to the long axes of the tooth. The specimens were polished for 20 s using a fine sharpening stone and etched for 20 seconds with 37% phosphoric acid (Classic Etch Gel) and mounted on aluminium stubs for analysis.

4.4.2. Scanning Electron Microscopy images

SEM micrographs of dentine and dentine-MTA interface of the samples that underwent element mapping were taken at various magnifications (100-1000x), and are presented in Figures 5.9-5.14.

4.4.3. Element mapping

Four randomly selected samples per group underwent element mapping using a SEM with EDX detector (Figure 4.4). The dentine-MTA interface and/or dentine (for groups with no MTA plug) were analysed at an accelerating voltage of 20 kV. Mapping of Ca, Si, P and O elements in the dentine-MTA interface and/or dentine were recorded. Energy Dispersive X ray Spectroscopy is an analytical technique that stimulates the emission of

characteristic x rays from a specimen by focusing upon it a beam of x rays. This excites electrons in the specimen bringing about the release of x rays with energies that are characteristic of the emitting element.

The mapping images for all samples were superimposed on the original SEM image and the MTA margin was marked using an Adobe® Photoshop (Adobe Systems, Inc., San Jose, CA, USA) tool.

Figure 4.4. Scanning Electron Microscope with Energy-dispersive X ray detector used in this study.

4.5. STATISTICAL ANALYSIS

The mean F-max and standard deviation (SD) were calculated for each group for the fracture resistance tests. D'Augusto and Pearson omnibus normality test revealed the data were normally distributed in all groups. These findings were analysed using one-way ANOVA with post hoc testing, by the Tukey's comparison of means test to localise significant differences. To give a measure of specimen dependability the fracture force (F) failure values for each group were fitted to the Weibull cumulative distribution function (Weibull 1951):

$$F(X) = 1 - e^{-[X/\alpha]^{\beta}}$$

where β is the Weibull modulus and α a measure of spread of the data and force at which 63.2% of specimens will fail, sometimes known also as the characteristic strength.

The Weibull modulus indicates the dependability of the specimens at low values of applied stress for failure at these levels will give a low opinion of a product's dependability. The higher the more dependable.

A Chi-square test was undertaken to compare the types (split and communited) of tooth fracture, and three depths of root fracture (above the cylinder, into the cylinder and vertical root fracture) seen across the groups. It tested the following null hypotheses:

- The experimental groups and the fracture types are independent
- The experimental groups and the fracture depths are independent.

The mean F-max and standard deviation were calculated for each type and depth of fracture. These findings were analysed using ANOVA test.

All statistical testing was performed to a level of statistical significance of $P < 0.05$.

The statistical packages for the tests were:

Fracture force

- ANOVA Package, GraphPad Prism, GraphPad Software Inc., La Jolla, CA, USA
- Tukey's (post hoc test) comparison of means test, GraphPad Prism, GraphPad Software Inc., La Jolla, CA, USA
- Weibull, Dental Materials Laboratory Program, University of Necastle upon Tyne, UK, 1985.

Mode of fracture

- Chi- square test, Preacher, K. J., 2001, Calculation for the chi-square test: An interactive calculation tool for chi-square tests of goodness of fit and independence [Computer software]. Available from http://quantpsy.org.
- ANOVA online calculation tool available from http://turner.faculty.swau.edu.

CHAPTER FIVE: RESULTS

5.1. Fracture resistance results

The raw collected fracture resistance results for reach group are presented in Tables 5.1-5.9.

Sample No.	F-max [N]	Fracture type		Fracture depth		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	459.1	✓				✓
2	169.1	✓			✓	
3	396.0	✓			✓	
4	300.7	✓			✓	
5	671.1	✓				✓
6	481.9	✓				✓
7	217.5	✓			✓	
8	404		✓		✓	
9	284.6	✓			✓	
10	330.2	✓		✓		
11	349.0	✓			✓	
12	366.4	✓			✓	
13	934.2	✓		✓		
14	248.3	✓				✓
15	790.6	✓		✓		
16	143.6	✓		✓		
17	606.7		✓		✓	
18	212.1	✓				✓
Mean F-max	**409.17**	**397.15**	**505.35**	**549.65**	**343.78**	**414.50**
SD	**211.52**	**218.51**	**101.35**	**323.64**	**119.18**	**167.90**

Table 5.1. Fracture resistance results for 12/52 CH + MTA(W) group.

Sample No.	F-max [N]	Fracture type		Fracture level		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	609.4	✓				✓
2	540.9		✓			✓
3	377.2	✓				✓
4	762.4		✓			✓
5	177.2	✓		✓		
6	530.2	✓				✓
7	817.4	✓		✓		
8	463.1	✓		✓		
9	374.4	✓				✓
10	275.2		✓		✓	
11	366.4	✓				
12	182.6	✓		✓		
13	291.3	✓			✓	✓
14	204	✓				✓
15	365.1	✓			✓	
16	357.1		✓		✓	
17	500.7	✓		✓		
18	845.6		✓		✓	
Mean F-max	**446.68**	**404.54**	**556.24**	**428.20**	**426.86**	**456.73**
SD	**201.07**	**175.31**	**221.41**	**237.18**	**212.32**	**163.12**

Table 5.2. Fracture resistance results for 2/52 CH + MTA(W) group.

Sample No.	F-max [N]	Fracture type		Fracture level		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	260.4	✓			✓	
2	324.8	✓			✓	
3	269.8		✓		✓	
4	851.0	✓			✓	
5	410.7		✓		✓	
6	296.7		✓		✓	
7	382.6		✓		✓	
8	461.7	✓			✓	
9	767.8	✓			✓	
10	801.3	✓		✓		
11	359.7		✓		✓	
12	271.1	✓			✓	
13	418.8	✓			✓	
14	421.5	✓			✓	
15	491.3	✓			✓	
16	307.4	✓			✓	
17	883.2	✓			✓	
18	342.3	✓			✓	
Mean F-max	**462.34**	**507.89**	**343.90**	**801.30**	**418.02**	
SD	**205.42**	**223.35**	**52.78**		**213.37**	

Table 5.3. Fracture resistance results for the Irrigation only group.

Sample No.	F-max [N]	Fracture type		Fracture level		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	247.0	✓			✓	
2	579.9	✓				✓
3	307.4	✓				✓
4	504.7	✓				✓
5	354.4	✓			✓	
6	426.9	✓				✓
7	232.2	✓				✓
8	402.7		✓		✓	
9	625.5	✓				✓
10	601.3		✓		✓	
11	1141		✓			✓
12	457.7	✓			✓	
13	1036		✓	✓		
14	569.1	✓			✓	
15	132.9		✓	✓		
16	336.9		✓		✓	
17	339.6	✓				✓
18	954.4		✓		✓	
Mean F-max	**513.87**	**387.71**	**657.89**	**587.45**	**490.44**	**462.41**
SD	**272.44**	**169.24**	**360.80**	**451.55**	**207.32**	**299.09**

Table 5.4. Fracture resistance results for 12/52 CH + MTA(PBS) group.

Sample No.	F-max [N]	Fracture type		Fracture level		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	847.0	✓		✓		
2	555.7	✓			✓	
3	828.2	✓			✓	
4	554.4	✓			✓	
5	358.4	✓		✓		
6	714.1		✓		✓	
7	613.4	✓			✓	
8	433.6	✓			✓	
9	687.2	✓		✓		
10	357.1	✓		✓		
11	583.9	✓		✓		
12	763.8	✓		✓		
13	397.3	✓		✓		
14	800.0	✓		✓		
15	394.6	✓		✓		
16	967.8	✓		✓		
17	324.8	✓		✓		
18	Fractured during	removal	from	an	acrylic	block
Mean F-max	**598.90**	**557.21**	**714.10**	**589.26**	**616.57**	**0**
SD	**194.00**	**236.80**		**222.81**	**126.07**	

Table 5.5. Fracture resistance results for 2/52 CH group.

Sample No	F-max [N]	Fracture type		Fracture level		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	590.6		✓			✓
2	903.4		✓		✓	
3	438.9	✓			✓	
4	934.2	✓		✓		
5	494.0	✓				✓
6	762.4	✓				✓
7	918.1		✓		✓	
8	998.7		✓		✓	
9	212.1		✓		✓	
10	300.7	✓				✓
11	809.4	✓			✓	
12	825.5		✓	✓		
13	872.5	✓			✓	
14	899.3	✓			✓	
15	436.2	✓				✓
16	1028	✓				✓
17	703.4	✓				✓
18	590.6		✓		✓	
Mean F-max	**706.56**	**650.34**	**719.86**	**879.85**	**738.00**	**616.47**
SD	**240.66**	**227.64**	**254.43**	**54.35**	**250.46**	**222.35**

Table 5.6. Fracture resistance results for 12/52 CH group.

Sample No.	F-max [N]	Fracture type		Fracture level		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	1250		✓	✓		
2	832.2	✓		✓		
3	1085	✓			✓	
4	655.0		✓			✓
5	1067	✓		✓		
6	1413		✓	✓		
7	283.2		✓		✓	
8	685.9	✓			✓	
9	1040	✓		✓		
10	783.9		✓	✓		
11	759.7		✓			✓
12	347.7		✓		✓	
13	1428		✓		✓	
14	814.8		✓			✓
15	906.0		✓		✓	
16	422.8	✓			✓	
17	459.1	✓			✓	
18	749.1	✓			✓	
Mean F-max	**832.36**	**794.63**	**898.68**	**1064.35**	**617.35**	**743.17**
SD	**328.72**	**246.18**	**359.65**	**219.53**	**267.24**	**66.27**

Table 5.7. Fracture resistance results for 2/52 CH + MTA(PBS) group.

Sample No.	F-max [N]	Fracture type		Fracture level		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	1400	✓				✓
2	566.4	✓			✓	
3	Fractured during	removal	from	an	acrylic	block
4	392.0	✓		✓		
5	1558	✓		✓		
6	914.1	✓		✓		
7	1157.0	✓				✓
8	975.8		✓		✓	
9	422.8		✓		✓	
10	598.7	✓				✓
11	426.9		✓		✓	
12	800.0	✓			✓	
13	747.4		✓		✓	
14	793.3		✓	✓		
15	1235	✓		✓		
16	636.2		✓			✓
17	1456		✓			✓
18	414.8	✓			✓	
Mean F-max	**852.61**	**903.60**	**779.77**	**978.48**	**622.01**	**1047.58**
SD	**375.7**	**395.84**	**331.56**	**395.99**	**205.76**	**369.33**

Table 5.8. Fracture resistance results for MTA(PBS) group.

Sample No.	F-max [N]	Fracture type		Fracture level		
		Split	Comminuted	Level of cylinder	Into the cylinder	VRF
1	1745	✓		✓		
2	1350	✓		✓		
3	1015	✓		✓		
4	1203		✓	✓		
5	1428		✓	✓		
6	1217	✓				✓
7	665.8		✓	✓		
8	365.1		✓		✓	
9	1133		✓			✓
10	1031	✓				✓
11	471.1	✓		✓		
12	226.9	✓			✓	
13	775.8	✓				✓
14	307.4	✓		✓		
15	989.3		✓	✓		
16	616.1	✓				✓
17	1004		✓			✓
18	1024		✓			✓
Mean F-max	**920.42**	**875.53**	**976.53**	**1019.40**	**296.00**	**971.56**
SD	**403.05**	**461.05**	**306.83**	**443.47**	**69.10**	**192.10**

Table 5.9. Fracture resistance results for MTA(W) group.

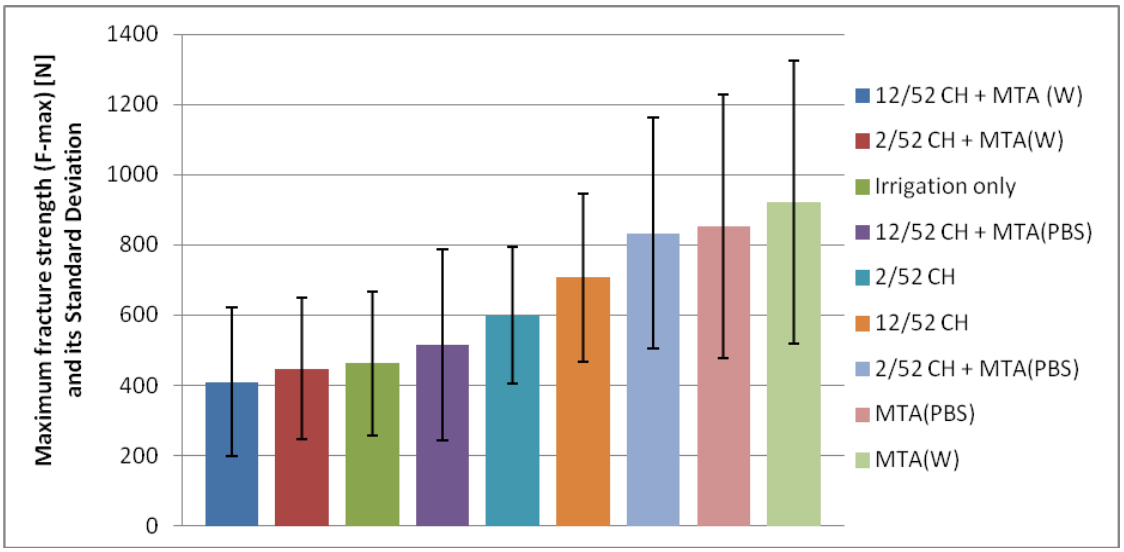
5.1.1. The mean maximum fracture strengths and their standard deviation

Table 5.10 and Figure 5.1 summarize the mean maximum fracture strengths (F-max) calculated for each group and their standard deviation.

Group	F-max [N]	Standard deviation
12/52 CH + MTA(W)	409.17	211.52
2/52 CH+ MTA(W)	446.68	201.07
IRRIGATION ONLY	462.34	205.42
12/52 CH + MTA(PBS)	513.87	272.44
2/52 CH	598.90	194.67
12/52 CH	706.56	240.66
2/52 CH + MTA(PBS)	832.36	328.73
MTA(PBS)	852.61	375.70
MTA(W)	920.41	403.05

CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline,
2/52 = dressed for two weeks, 12/52 =dressed for 12 weeks.

Table 5.10. Summary of the mean maximum fracture strengths (F-max) calculated for each group and their standard deviation.



CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline,
2/52 = dressed for two weeks, 12/52 =dressed for 12 weeks.

Figure 5.1. Summary of the mean maximum fracture strengths (F-max) calculated for each group and their standard deviation.

5.1.2. The ANOVA test results

Prior to conducting the ANOVA all data was confirmed normally distributed by D'Agustino and Pearson omnibus normality test. The result of the one-way ANOVA test for the mean F-max between all groups showed highly statistically significant difference among the groups, with $P < 0.0001$.

5.1.3. The Tukey's comparisons of F-max means results

The ANOVA results were localised by a Tukey comparison of means test (Table 5.11) that highlighted significant differences between groups:

- 12/52 CH +MTA(W) vs 12/52 CH,
  2/52 CH + MTA(PBS),
  MTA(PBS),
  MTA(W)
- 2/52 CH + MTA(W) vs 2/52 CH + MTA(PBS),
  MTA(PBS),
  MTA(W)
- IRRIGATION ONLY vs 2/52 CH + MTA(PBS),
  MTA(PBS),
  MTA(W)
- 12/52 CH + MTA(PBS) vs 2/52 CH + MTA(PBS),
  MTA(PBS),
  MTA(W)
- 2/52 CH vs MTA(W)

	2/52 CH + MTA(W)	Irrigation only	12/52 CH + MTA(PBS)	2/52 CH	12/52 CH	2/52 CH + MTA(PBS)	MTA(PBS)	MTA(W)
12/52 CH +MTA(W)	NS	NS	NS	NS	*	***	****	****
2/52 CH + MTA(W)	-	NS	NS	NS	NS	**	***	****
IRRIGATION ONLY	-	-	NS	NS	NS	**	**	****
12/52 CH + MTA(PBS)	-	-	-	NS	NS	*	*	***
2/52 CH	-	-	-	-	NS	NS	NS	*
12/52 CH	-	-	-	-	-	NS	NS	NS
2/52 CH + MTA(PBS)	-	-	-	-	-	-	NS	NS
MTA(PBS)	-	-	-	-	-	-	-	NS

NS = Not significant, * = $P < 0.05$, ** = $P < 0.01$, *** = $P < 0.001$, **** = $P < 0.0001$.

CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 =dressed for 12 weeks.

Table 5.11. Tukey's comparison of means summary.

5.1.4. The Weibull moduli results

The outcome of a Weibull analysis of the fracture strength data is given in Table 5.12. This gives for each group the number of specimens tested (n), the goodness of fit of the data to the Weibull distribution (R), the Weibull modulus and its standard error (S.E.) together with the value of force at which 63.2% of specimens would fail (α). Table 5.13 gives a summary of a comparison of the Weibull moduli.

Figures 5.2 and 5.3 illustrate the probability of failure in relation to applied load for each experimental group.

There are statistically significant differences in ($P < 0.05$) the Weibull moduli (dependability of the specimen to fracture) between the following groups:

- 12/52 CH vs 12/52 CH + MTA(W),
  2/52 CH + MTA(W),
  12/52 CH + MTA(PBS)
- 2/52 CH + MTA(PBS) vs 2/52 CH,
- MTA(W) vs 2/52 CH + MTA(W),
  2/52 CH,
  2/52 CH + MTA(PBS),
  MTA(PBS)

The Weibull moduli analysis confirmed the higher dependability of samples in group (Tables 5.11):

- 12/52 CH vs 12/52 CH + MTA(W),
- MTA(W) vs 2/52 CH + MTA(W),

  2/52 CH

as shown by the Tukey comparison of means of the fracture strength data (Table 5.11) but also flagged up, missed by the Tukey analysis of fracture strength data, issues concerning higher dependability of:

- 12/52 CH vs 2/52 CH + MTA(W) and

  12/52 CH + MTA(PBS) (Figure 5.4)
- 2/52 CH +MTA(PBS) vs 2/52 CH (Figure 5.5),

and poorer dependability of:

- MTA(W) vs 2/52 CH + MTA(PBS) and

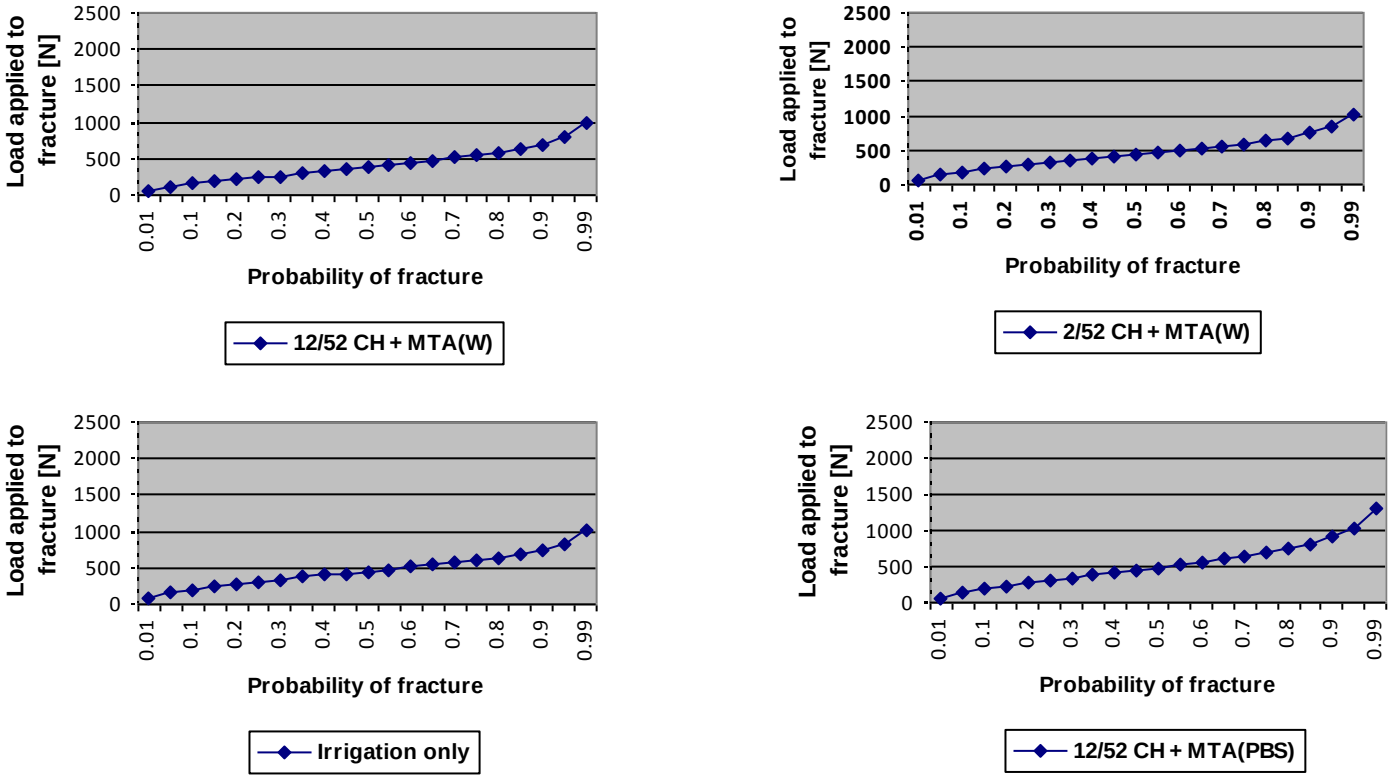
  MTA(PBS) (Figure 5.6)

  at higher failure probability of values.

Group	R	N	Weibull modulus (S.E.)	Characteristic strength the force at which 63.2% of specimens will fail (α)
12/52 CH + MTA(W)	0.96	18	2.04 (0.11)	465.23
2/52 CH+ MTA(W)	0.96	18	2.20 (0.12)	509.57
IRRIGATION ONLY	0.81	18	2.38 (0.28)	528.30
12/52 CH + MTA(PBS)	0.96	18	1.93 (0.10)	586.80
2/52 CH	0.94	17	2.97 (0.19)	674.15
12/52 CH	0.95	18	2.37 (0.14)	813.52
2/52 CH + MTA(PBS)	0.98	18	2.31 (0.10)	951.82
MTA(PBS)	0.93	17	2.22 (0.16)	974.25
MTA(W)	0,97	18	1.87 (0.01)	1070.00

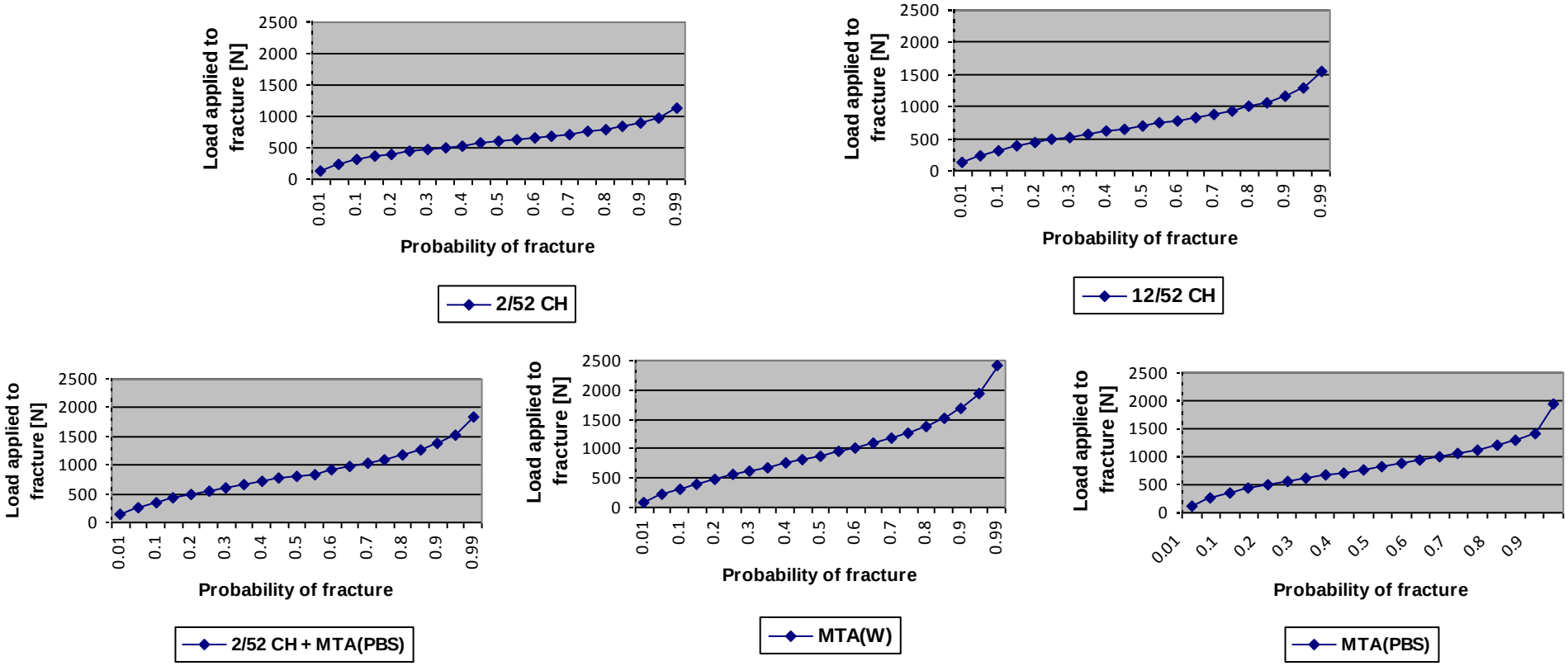
CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 = dressed for 12 weeks.

Table 5.12. Summary of the Weibull moduli.



CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 = dressed for 12 weeks.

Figure 5.2. The charts illustrate the probability of failure in relation to the applied load [N] for groups 12/52 CH+MTA(W), 2/52 CH+MTA(W), Irrigation only and 12/52 CH+ MTA(PBS).



CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 = dressed for 12 weeks.

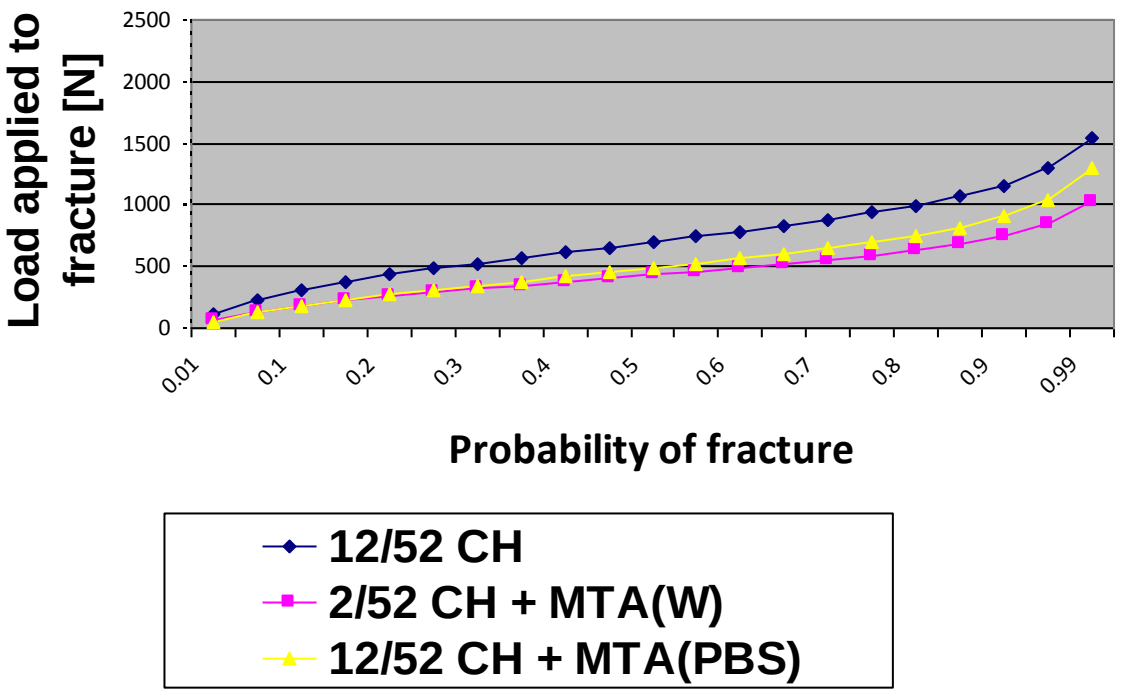
Figure 5.3. The charts illustrate the probability of failure in relation to the applied load [N] for groups 2/52 CH, 12/52 CH, 2/52 CH + MTA(PBS), MTA(PBS) and MTA(W).

Group versus	2/52 CH + MTA(W)	Irrigation only	12/52 CH + MTA(PBS)	2/52 CH	12/52 CH	2/52 CH + MTA(PBS)	MTA(PBS)	MTA(W)
12/52 CH +MTA(W)	NS	NS	NS	NS	*	NS	NS	NS
2/52 CH + MTA(W)	-	NS	NS	NS	*	NS	NS	*
IRRIGATION ONLY	-	-	NS	NS	NS	NS	NS	NS
12/52 CH + MTA(PBS)	-	-	-	NS	*	NS	NS	NS
2/52 CH	-	-	-	-	NS	*	NS	*
12/52 CH	-	-	-	-	-	NS	NS	NS
2/52 CH + MTA(PBS)	-	-	-	-	-	-	NS	*
MTA(PBS)	-	-	-	-	-	-	-	*

NS = Not significant, * = $P < 0.05$, highlighted =confirmed by Turkey comparison of means.

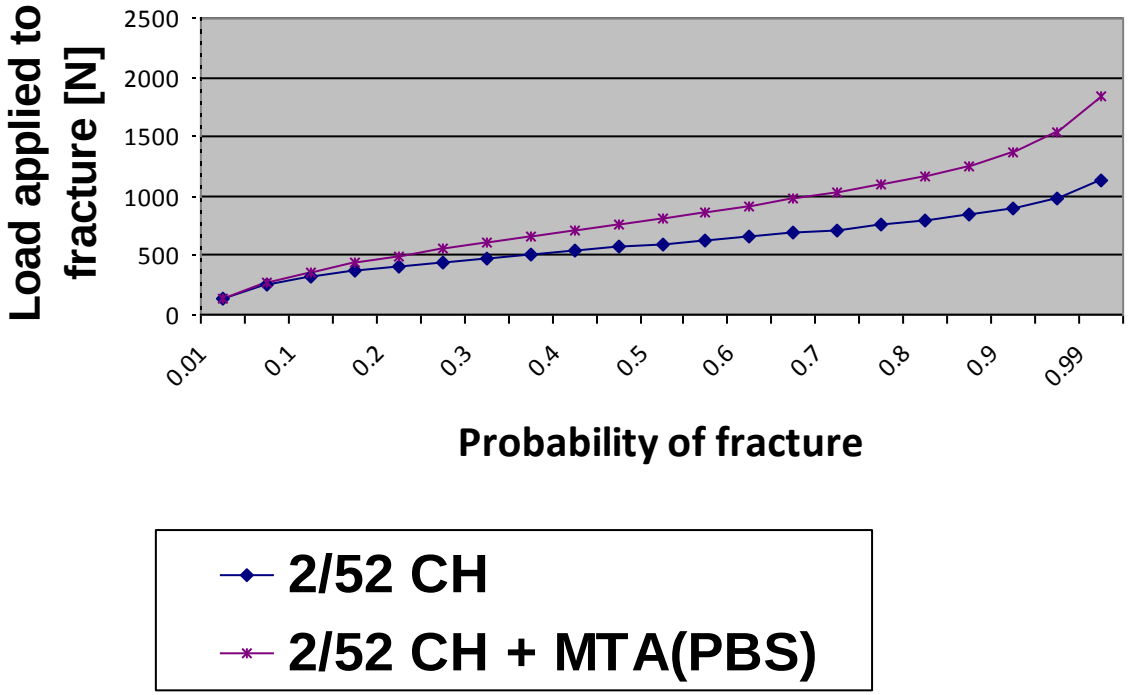
CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W)= MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 =dressed for 12 weeks.

Table 5.13. Summary of a comparison of the Weibull moduli.



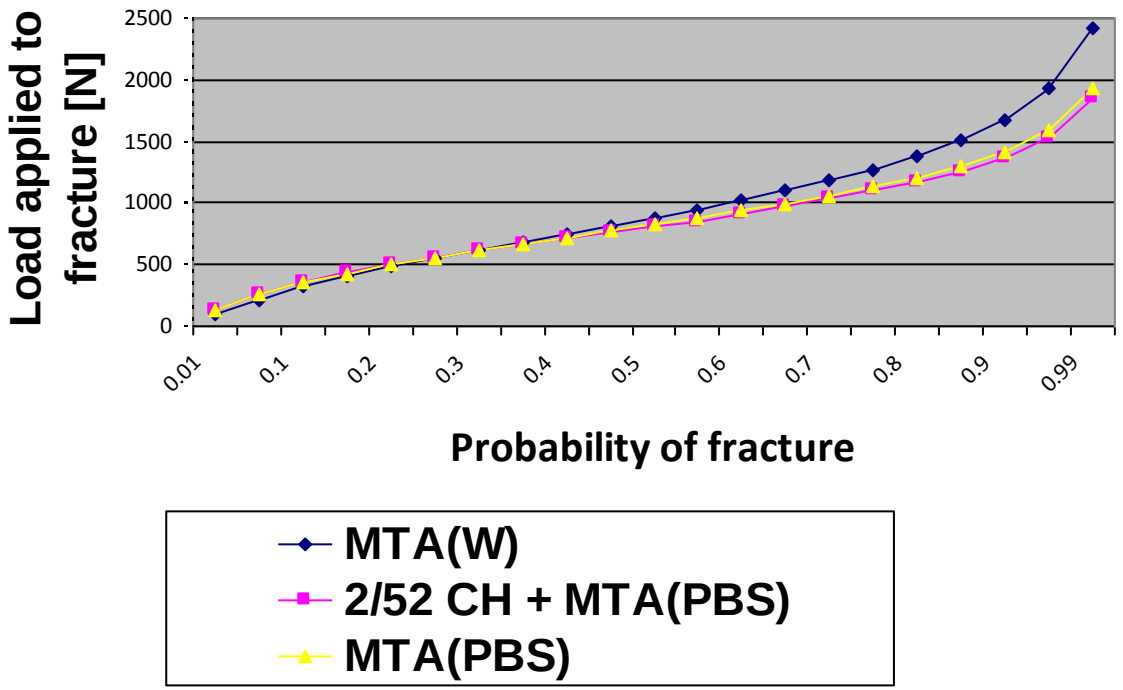
CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 = dressed for 12 weeks.

Figure 5.4. The chart illustrates significant differences between the probability of failure in relation to the applied load [N] between groups: 12/52 CH versus 2/52 CH + MTA(W) and 12/52 CH + MTA(PBS).



CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks.

Figure 5.5. The chart illustrates significant differences between the probability of failure in relation to the applied load [N] between groups: 2/52 CH+ MTA(PBS) versus 2/52 CH.



CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks.

Figure 5.6. The chart illustrates significant differences between the probability of failure in relation to the applied load [N] between groups: MTA(W) versus 2/52 CH + MTA(PBS) and MTA(PBS). Note almost identical behaviour of 2/52 CH + MTA(PBS) and MTA(PBS) groups.

5.1.5. The Chi-square calculation results

The summary of the numbers and percentages of root fracture types (split and comminuted) encountered within each group are summarised in Table 5.14.The Chi-square calculation of the two modes of tooth fracture, revealed Chi-square value of 19.06, with *P* value of 0.015, and rejected the null hypothesis that the experimental groups and the fracture modes are independent (Figure 5.7).

The summary of the numbers and percentages of three fracture depths (above the cylinder, into the cylinder and vertical root fracture) encountered within each group are summarised in Table 5.15. Chi-square calculation of the three depths of root fracture, revealed Chi-square value of 50.46, *P* value of 0.00002 (Figure 5.7), which rejected the null hypothesis that the experimental groups and the fracture depths were independent.

Therefore, from both Chi-square tests it is apparent that the treatment of the root influences the mode of fracture.

Group of roots	Split		Comminuted	
	Number [%]	Mean F-max (N)	Number [%]	Mean F-max (N)
12/52 CH + MTA(W)	17 [54]	397.15	1 [6]	505.35
2/52 CH + MTA(W)	12 [67]	404.54	6 [33]	556.24
Irrigation only	14 [78]	507.89	4 [22]	343.90
12/52 CH + MTA(PBS)	12 [67]	387.71	6 [33]	657.89
2/52 CH	16 [94]	557.21	1 [6]	714.10
12/52 CH	11 [61]	650.34	7 [39]	719.86
2/52 CH + MTA(PBS)	8 [44]	794.63	10 [56]	898.68
MTA(PBS)	10 [59]	903.60	7 [41]	779.77
MTA(W)	10 [56]	875.53	8 [44]	976.53
Total [SD]	**110 [69]**	**608.73 [195.09]**	**50 [31]**	**683.59 [184.87]**

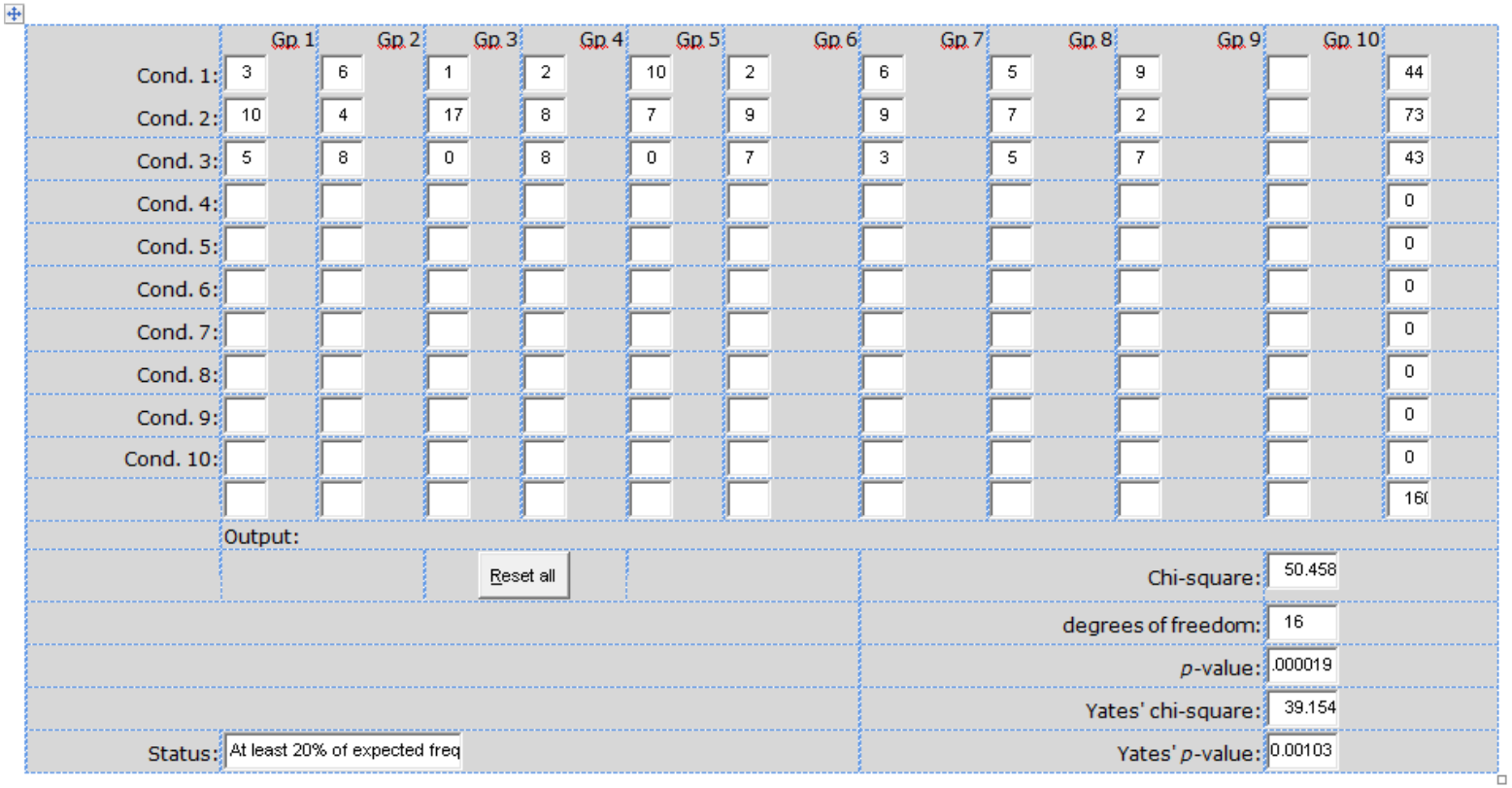
CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 =dressed for 12 weeks, SD = Standard Deviation.

Table 5.14.The numbers and percentages of fracture types encountered within each group of roots, their mean F-max and standard deviation.

Group of roots	Cylinder level		Below cylinder level		VRF	
	Number [%]	Mean F-max (N)	Number [%]	Mean F-max (N)	Number [%]	Mean F-max (N]
12/52 CH + MTA(W)	3 [17]	549.65	10 [56]	343.78	5 [28]	414.50
2/52 CH + MTA(W)	6 [33]	428.20	4 [22]	426.86	8 [44]	456.73
Irrigation only	1 [6]	801.30	17 [94]	418.02	0 [0]	0
12/52 CH + MTA(PBS)	2 [11]	584.45	8 [44]	490.44	8 [44]	462.41
2/52 CH	10 [59]	589.26	7 [41]	616.57	0 [0]	0
12/52 CH	2 [11]	879.85	9 [50]	738.00	7 [39]	616.47
2/52 CH + MTA(PBS)	6 [33]	1064.35	9 [50]	617.35	3 [17]	743.17
MTA(PBS)	5 [29]	978.48	7 [39]	622.01	5 [29]	1047.58
MTA(W)	9 [50]	1019.40	2 [11]	296.00	7 [39]	971.56
Total [SD]	**44 [27.5]**	**766.10 [220.53]**	**73 [45]**	**507.67 [140.06]**	**43 [27]**	**673.20 [273.70]**
			116 [72.5%]		**575.12 [143.23]**	

CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 = dressed for 12 weeks, SD = Standard Deviation.

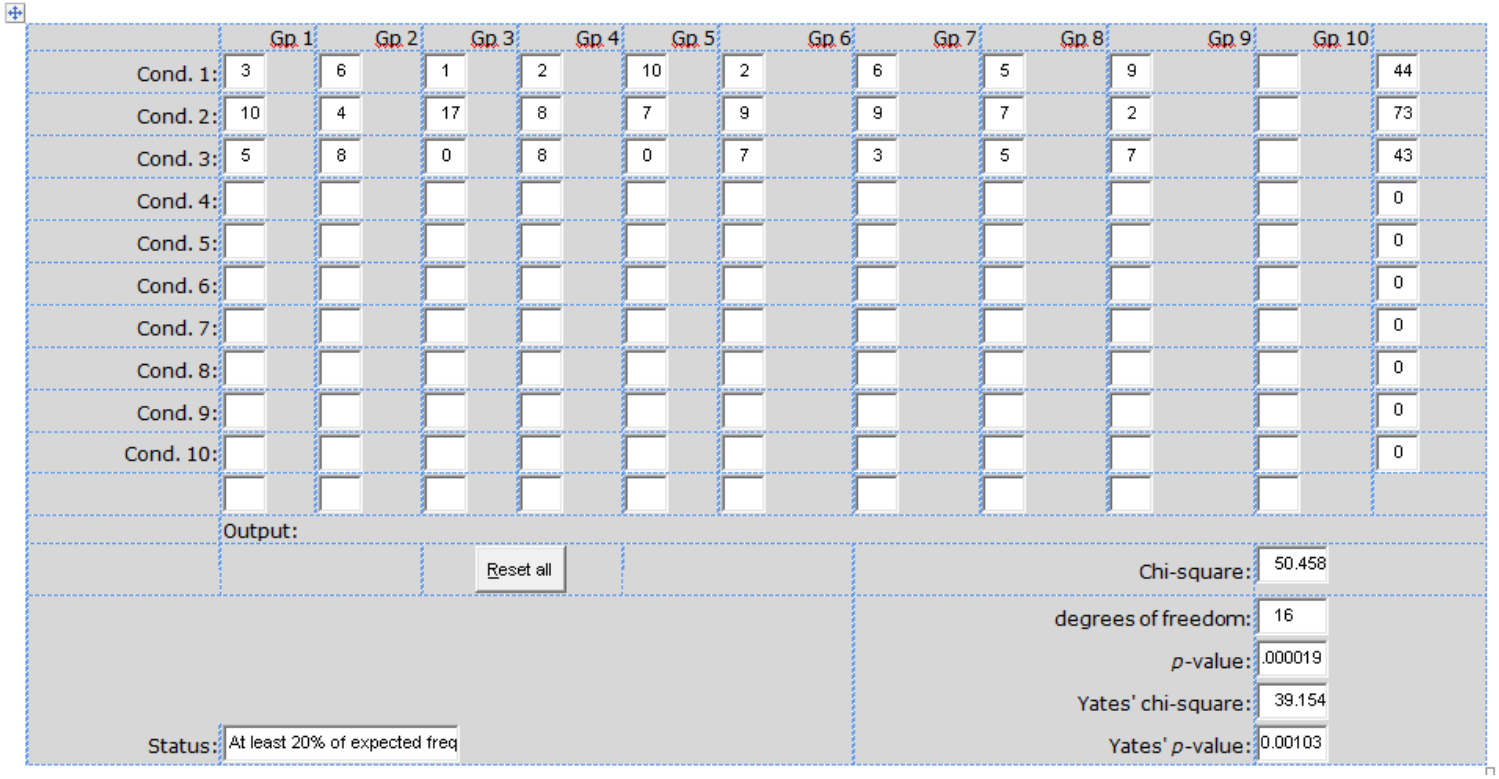
Table 5.15. The numbers and percentages of fracture depths encountered within each group of roots, their mean F-max and standard deviation.



Gp 1: 12/52 CH + MTA(W), Gp 2: 2/52 CH + MTA (W), Gp 3: Irrigation only, Gp 4: 12-52 CH + MTA (PBS), Gp 5: 2/52 CH, Gp 6: 12/52 CH, Gp 7: 2/52 CH +MTA (PBS), Gp 8: MTA (PBS), Gp 9: MTA(W). Cond. 1: Split type of fracture, Cond. 2: Comminuted type of fracture.

CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 = dressed for 12 weeks.

Figure 5.7. Chi-square calculation of two modes of tooth fracture in all groups.



	Gp 1	Gp 2	Gp 3	Gp 4	Gp 5	Gp 6	Gp 7	Gp 8	Gp 9	Gp 10	
Cond. 1:	3	6	1	2	10	2	6	5	9		44
Cond. 2:	10	4	17	8	7	9	9	7	2		73
Cond. 3:	5	8	0	8	0	7	3	5	7		43
Cond. 4:											0
Cond. 5:											0
Cond. 6:											0
Cond. 7:											0
Cond. 8:											0
Cond. 9:											0
Cond. 10:											0

Output:

Reset all

Chi-square: 50.458

degrees of freedom: 16

p-value: .000019

Yates' chi-square: 39.154

Yates' *p*-value: 0.00103

Status: At least 20% of expected freq

Gp 1: 12/52 CH + MTA(W), Gp 2: 2/52 CH + MTA (W), Gp 3: Irrigation only, Gp 4: 12-52 CH + MTA (PBS), Gp 5: 2/52 CH, Gp 6: 12/52 CH, Gp 7: 2/52 CH +MTA (PBS), Gp 8: MTA (PBS), Gp 9: MTA(W). Cond. 1: Fractured at cylinder level, Cond. 2: Fractured below cylinder level, Cond.3: Vertical root fracture.

CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS)= MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 = dressed for 12 weeks.

Figure 5.8. Chi-square calculation of three depths of tooth fracture in all groups.

5.1.6. The ANOVA test result for the mean F-max for the two fracture types

The result of the one-way ANOVA test for the mean F-max between two fracture types (split vs comminuted) showed no statistically significant difference between the mean F-max for two fracture types (*P* = 0.7920).

Comparison of means for two fracture type modalities

	Split	Comminuted
1	397.15	505.35
2	404.54	556.24
3	507.89	343.9
4	387.71	657.89
5	557.21	714.1
6	650.34	719.86
7	794.63	898.68
8	903.6	779.77
9	875.53	976.53
n	9	9
$\overline{X}$	608.733	683.591
s	206.928	196.090
$\overline{X}_{ave}$	646.162	

source	df	SS	MS	F	P-value
treatments	1	25216.591	25216.591	0.6206	0.7920
error	16	650165.091	40635.318		
total	17	675381.682			

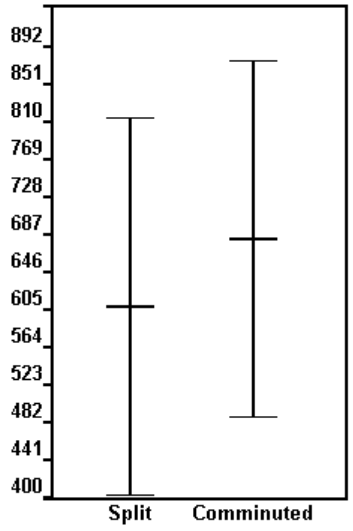


Figure 5.9. Summary of the ANOVA test calculation for the mean F-max for the two fracture depth types.

Graph displays the mean for each group and a vertical “error bar” containing values within one standard deviation of the mean.
http://turner.faculty.swau.edu.

5.1.7. The ANOVA test results for the mean F-max for three fracture depths

The result of the one-way ANOVA test for the mean F-max between three fracture depths (above the cylinder vs into the cylinder vs VRF) showed no statistically significant difference between the fracture depths (*P* = 0.0538).

Comparison of means for three fracture depth modalities

	Level of cylinder	Into cylinder	VRF
1	549.65	343.78	414.5
2	428.2	426.86	456.73
3	584.45	490.44	462.41
4	879.85	738	616.47
5	1064.35	617.35	743.17
6	978.48	622.01	1047.58
7	1019.4	296	971.56
8	589.26	616.57	
9	801.3	418.02	
n	9	9	7
$\bar{X}$	766.104	507.670	673.203
s	233.905	148.553	256.746
$\bar{X}_{ave}$	647.056		

source	df	SS	MS	F	P-value
treatments	2	307194.509	153597.255	3.3465	0.0538
error	22	1009746.812	45897.582		
total	24	1316941.321			

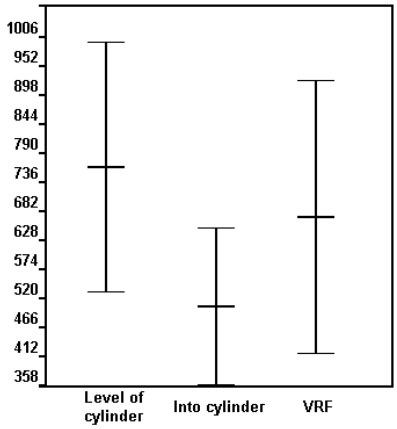


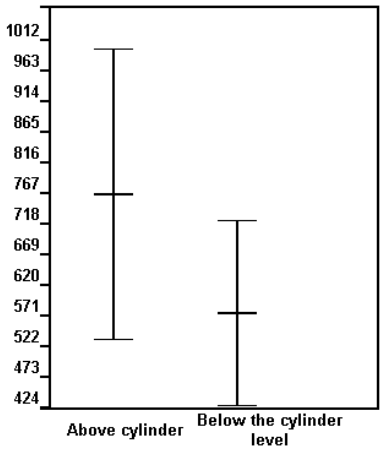
Figure 5.10. Summary of the ANOVA test calculation for the mean F-max for the three fracture types.

Graph displays the mean for each group and a vertical “error bar” containing values within one standard deviation of the mean.
http://turner.faculty.swau.edu.

5.1.8. The ANOVA test results for the mean F-max for fracture above and below the cylinder

The result of the one-way ANOVA test for the mean F-max between fracture depths: above the cylinder vs below the cylinder showed no statistically significant difference between the fracture modalities (P = 0.3044).

	Above cylinder	Below the cylinder level
1	549.65	397.14
2	428.20	441.80
3	801.3	418.02
4	584.45	476.43
5	589.26	616.57
6	879.85	677.24
7	1064.35	680.26
8	978.48	834.80
9	1019.4	633.78
n	9	9
$\overline{X}$	766.104	575.116
s	233.905	149.142
$\overline{X}_{ave}$	670.610	



source	df	SS	MS	F	P-value
treatments	1	164145.401	164145.401	4.2660	0.3044
error	16	615640.383	38477.524		
total	17	779785.784			

Figure 5.11. Summary of the ANOVA test calculation for the mean F-max for fractures above and below the cylinder.

Graph displays the mean for each group and a vertical “error bar” containing values within one standard deviation of the mean.
http://turner.faculty.swau.edu.

5.2. ELEMENT MAPPING RESULTS

5.2.1. The SEM examination results

The scanning electron microscopy images of the groups restored with water- and PBS-mixed MTA are presented in Figures 5.9-5.14.

In the SEM examination of groups filled with MTA mixed with PBS, it was possible to distinguish the interfacial layer between the cement and dentine, and a 50-200 μm layer of altered dentine adjacent to MTA. No such findings could be noted in groups restored with MTA mixed with water.

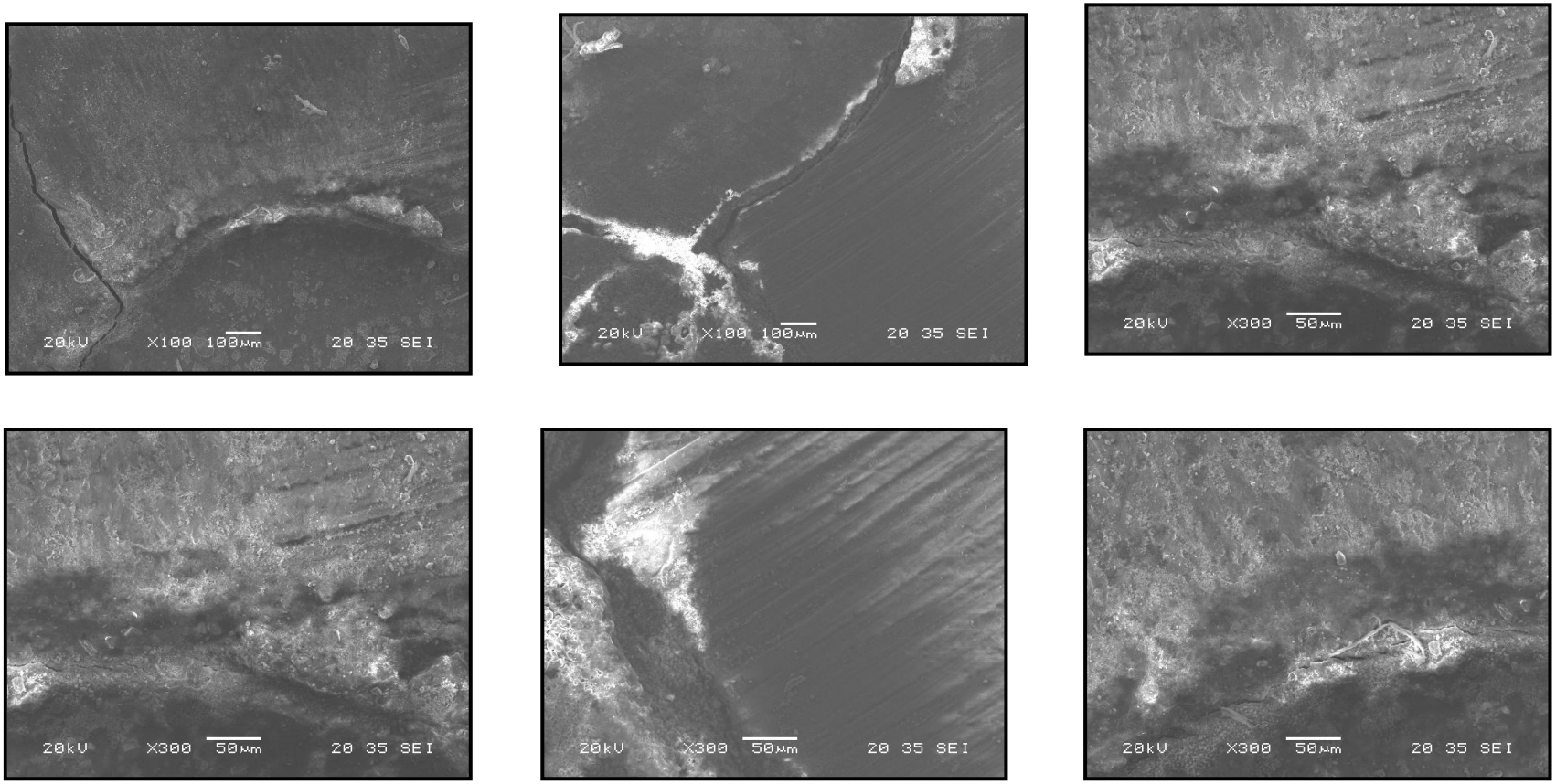


Figure 5.9. The Scanning Electron Microscopy images of group 12/52 CH + MTA(W).

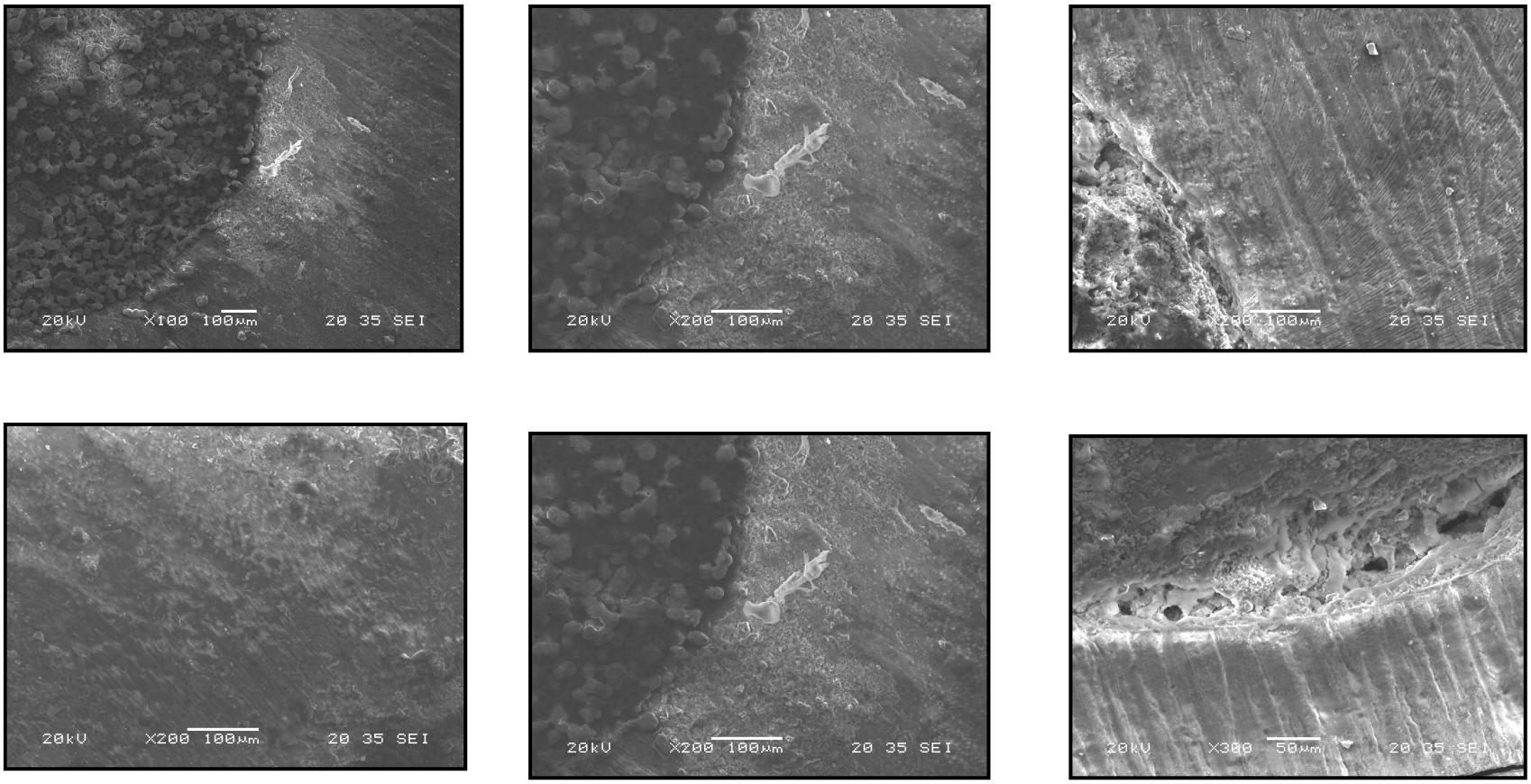


Figure 5.10. The Scanning Electron Microscopy images of group 2/52 CH + MTA(W).

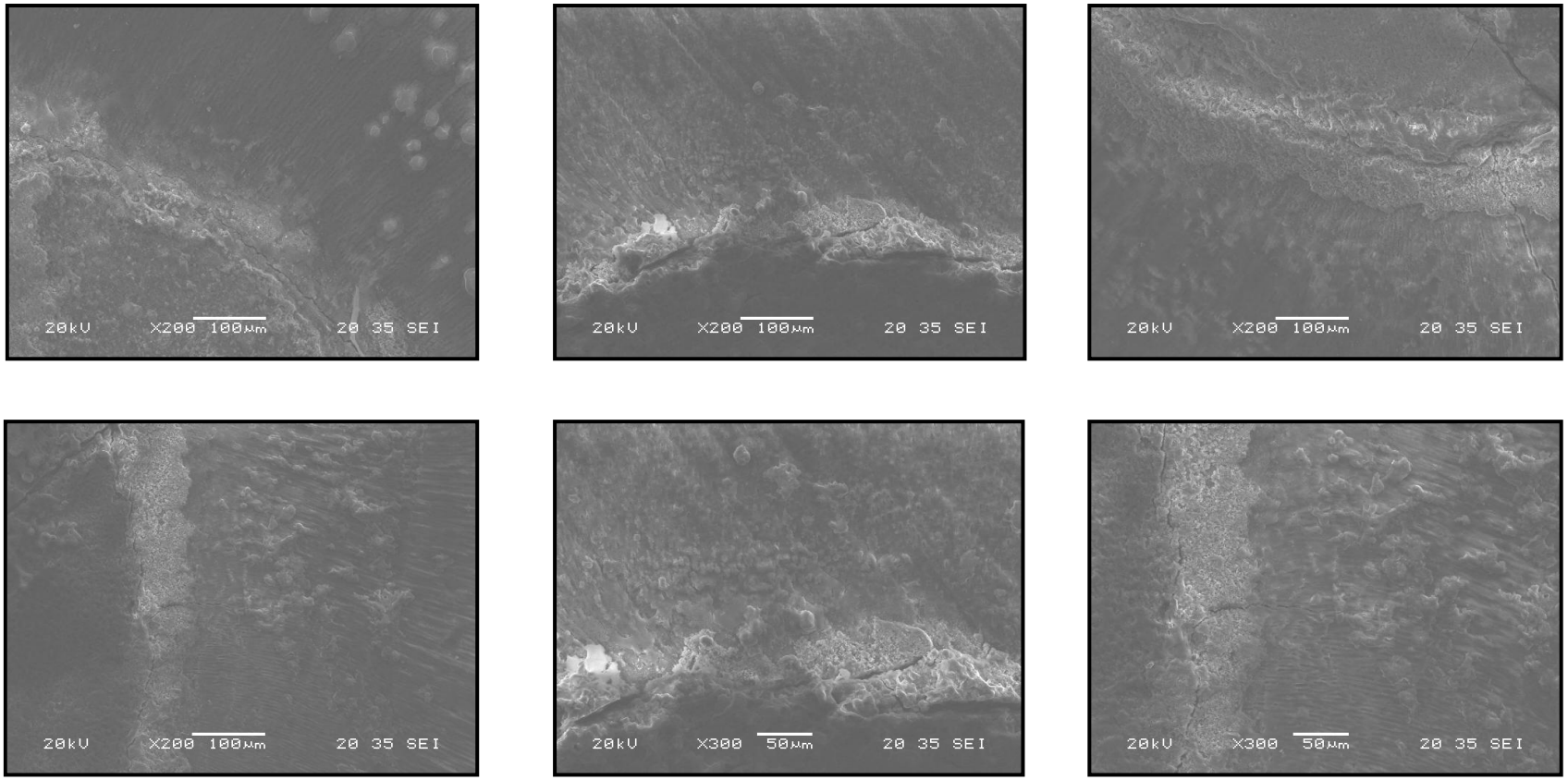


Figure 5.11. The Scanning Electron Microscopy images of group 12/52 CH + MTA(PBS).

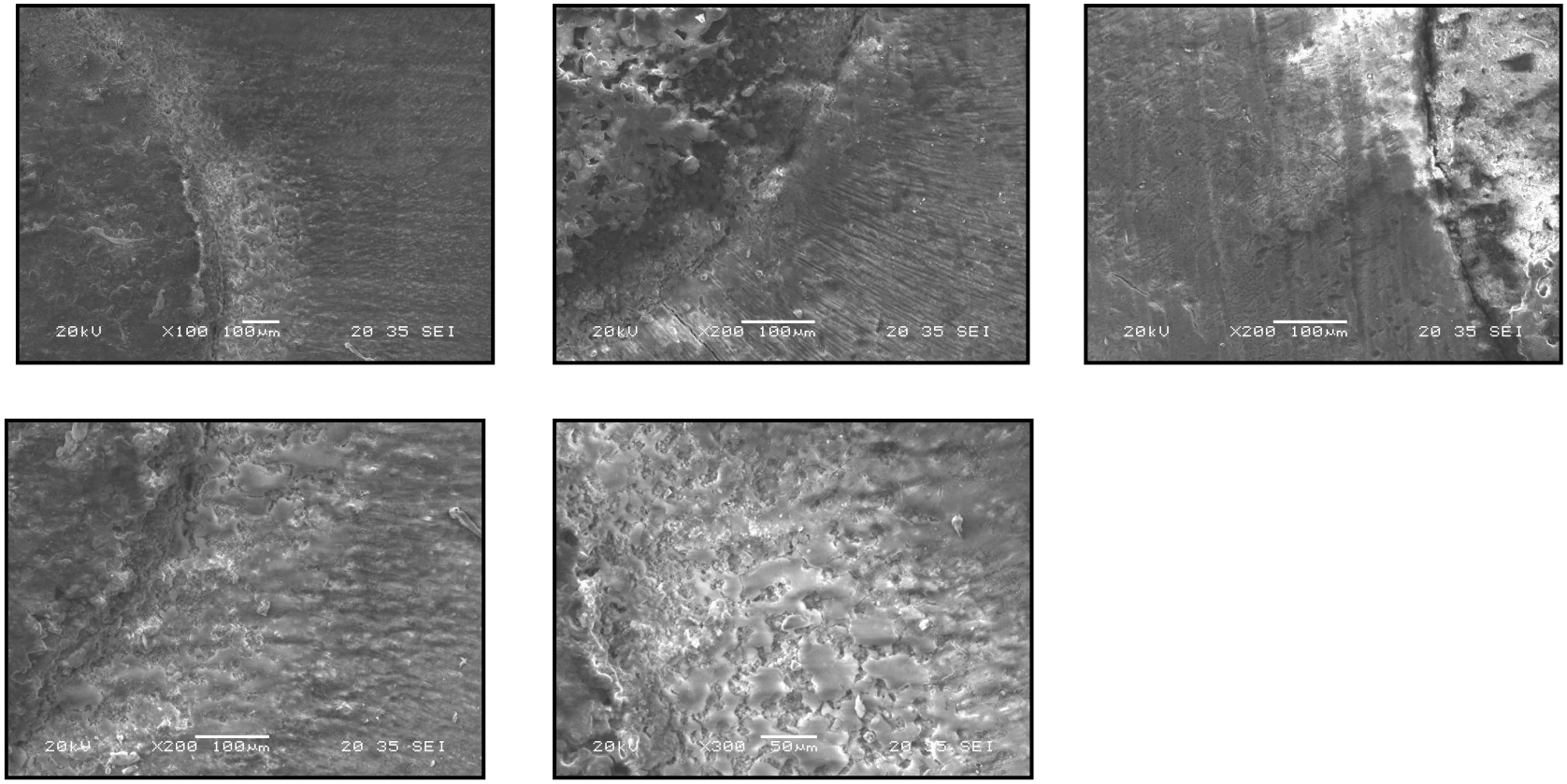


Figure 5.12. The Scanning Electron Microscopy images of group 2/52 CH + MTA(PBS).

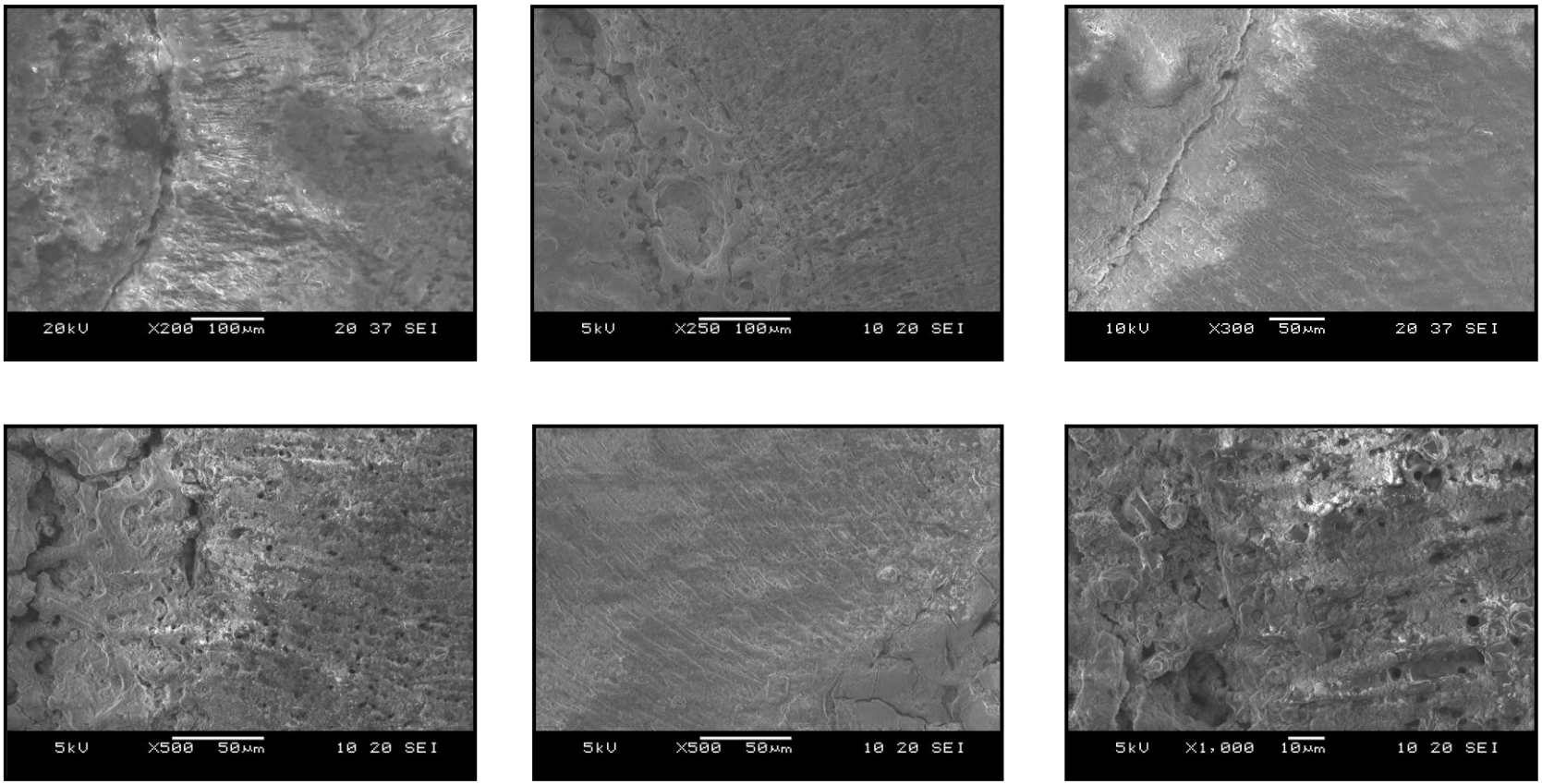


Figure 5.13. The Scanning Electron Microscopy images of group MTA(PBS).

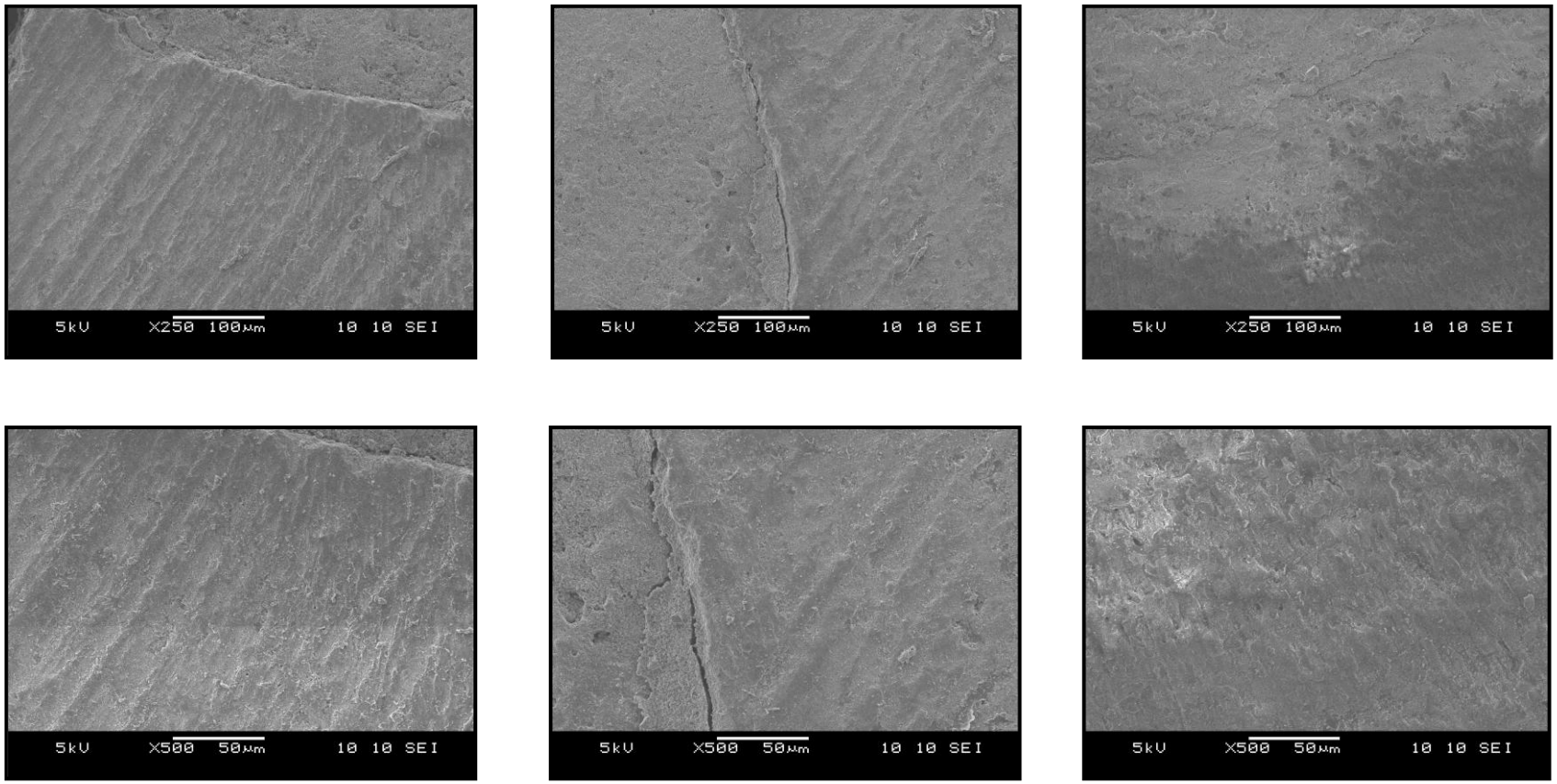


Figure 5.14. The Scanning Electron Microscopy images of group MTA(W).

5.2.2. The element mapping results

Element (Ca, Si, P and O) mapping results of the dentine-MTA interface and / or the dentine for each group are presented in Figures 5.15 - 5.48.

The summary of element diffusion into dentine is presented in Table 5.16.

Group	Element diffusion into dentine [maximum depth of diffusion]
12/52 CH +MTA(W)	None, dentine depleted of P up to 100 μm
2/52 CH + MTA(W)	None
IRRIGATION ONLY	None
12/52 CH + MTA(PBS)	Si [100 μm], P [100 μm], O [100 μm], Ca [50 μm]
2/52 CH	None
12/52 CH	None
2/52 CH + MTA(PBS)	Si [350 μm], P [100 μm], Ca [50 μm], O [300 μm]
MTA(PBS)	Si [150 μm]
MTA(W)	Si [170 μm], P [100 μm], O [170 μm], Ca [100 μm],

CH = Calcium hydroxide, MTA = Mineral Trioxide Aggregate, MTA(W) = MTA mixed with water, MTA(PBS) = MTA mixed with phosphate-buffered saline, 2/52 = dressed for two weeks, 12/52 = dressed for 12 weeks.

Table 5.16. Summary of element diffusion and depths of diffusion for all groups.

For the element mapping it is clear that there is Si, P, Ca, O element diffusion from the MTA root fillings into dentine in groups MTA(W), 2/52 CH+ MTA(PBS) and 12/52 CH+ MTA(PBS). Only Si diffusion could be observed in MTA(PBS) group. Up to 100 μm band of P-depleted dentine adjacent to MTA could be observed in group 12/52 CH + MTA(W).

No element diffusion could be detected in groups: Irrigation only, 2/52 CH, 12/52 CH, 2/52 CH + MTA(W) and 12/52 CH + MTA(W).

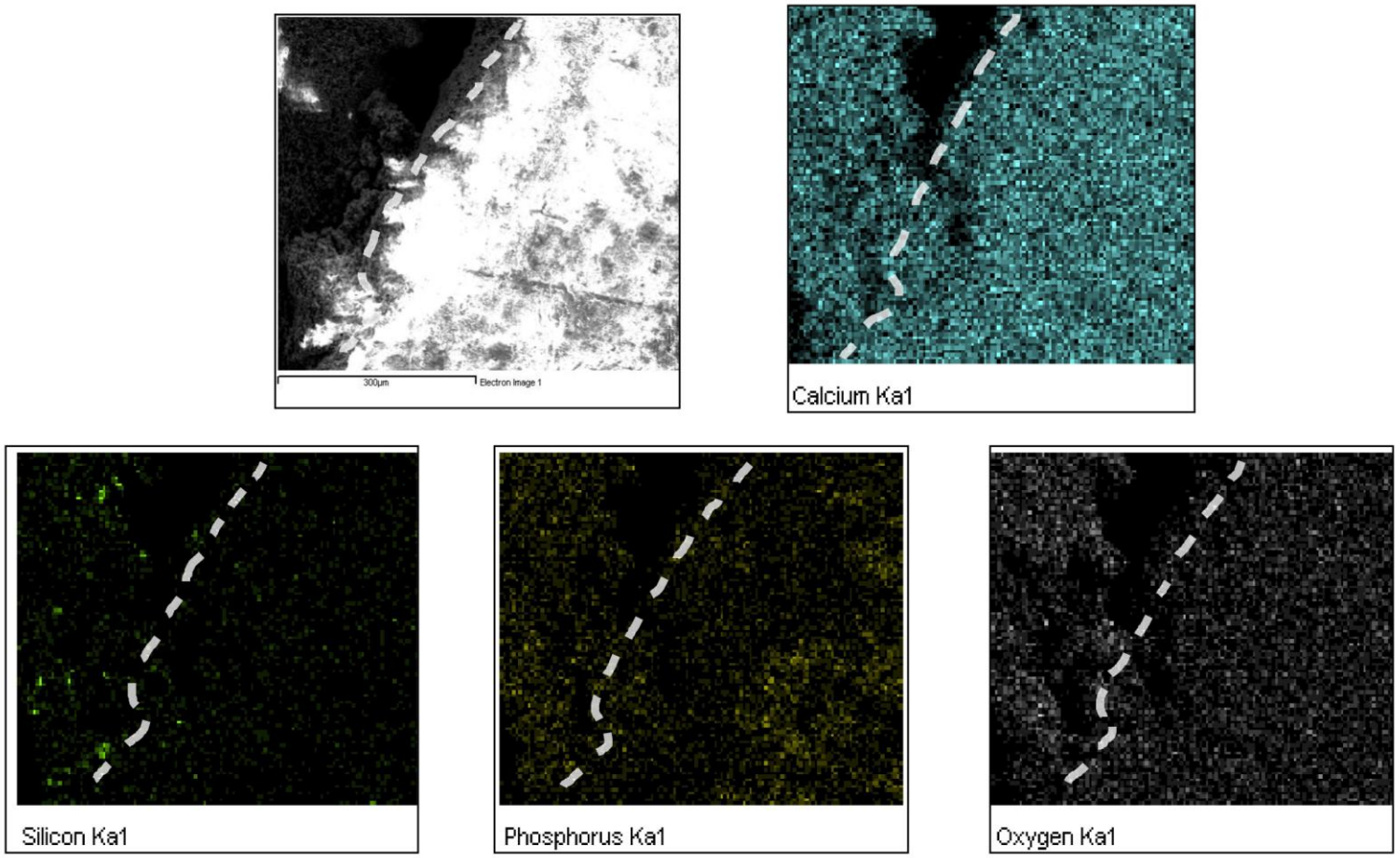


Figure 5.15. Mapping results for group 12/52 CH + MTA(W), mapping No. 1. Dotted line shows the approximate margin of the MTA.

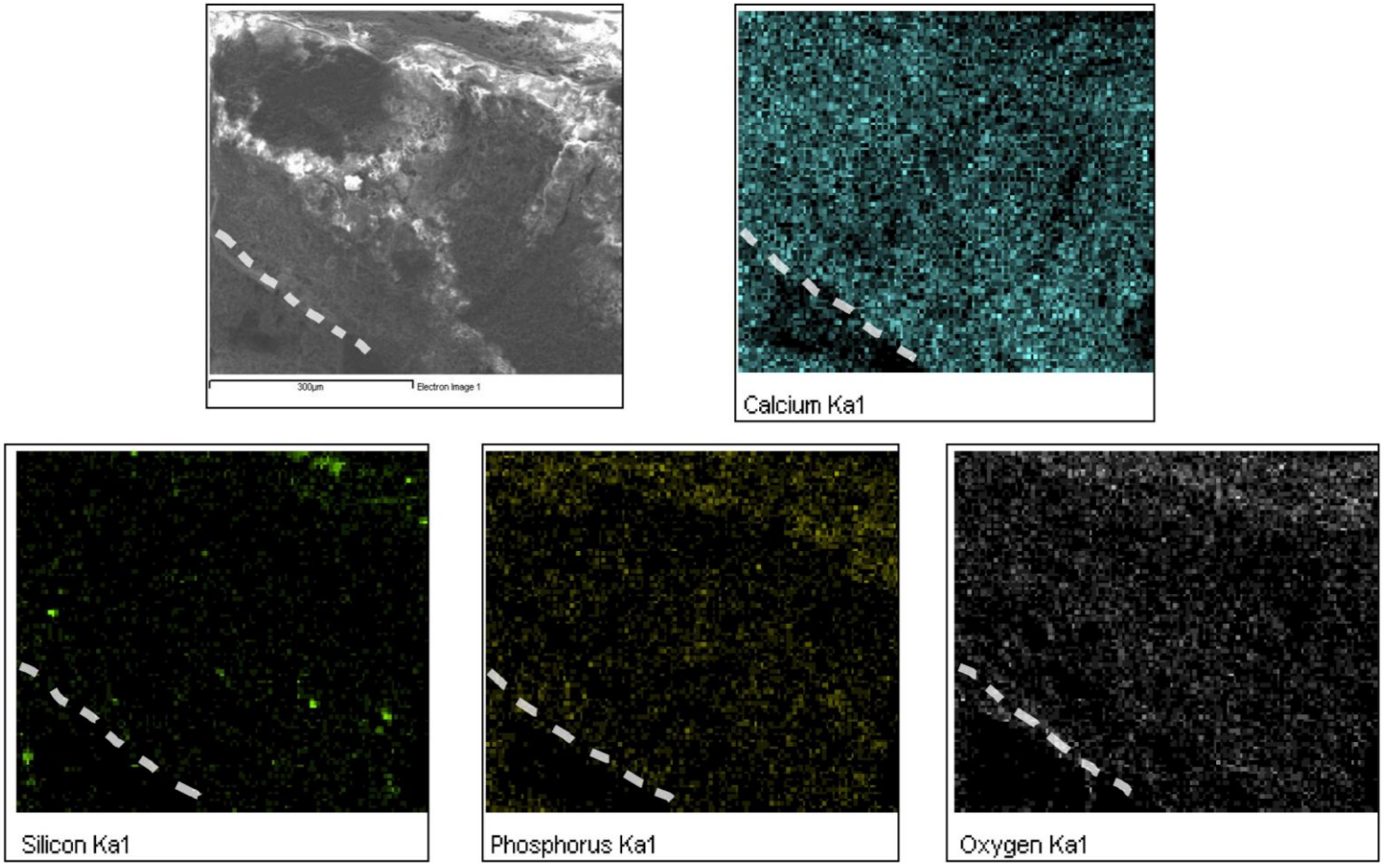


Figure 5.16. Mapping results for group 12/52 CH + MTA(W), mapping No. 2. Dotted line shows the approximate margin of the MTA.

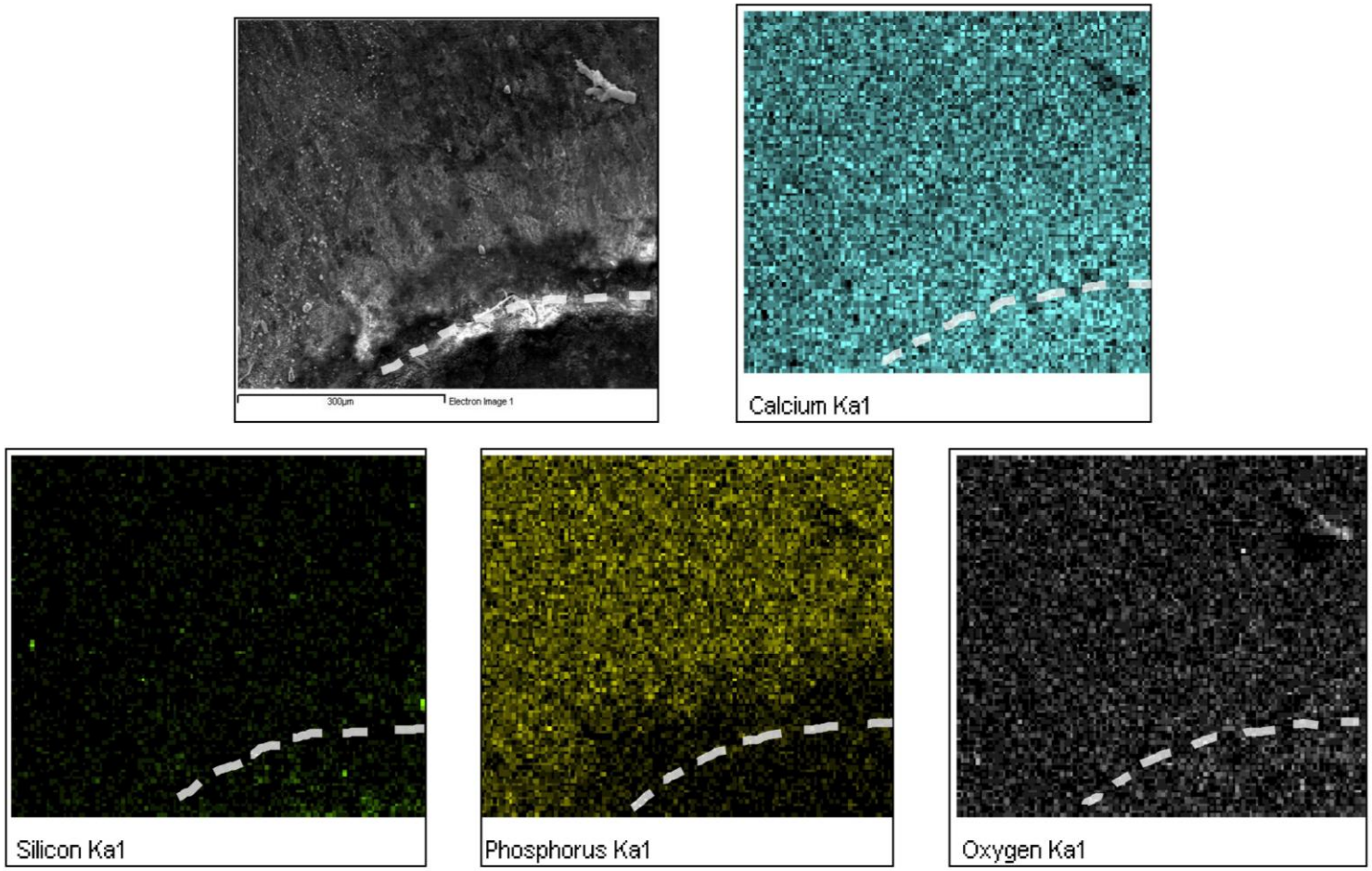


Figure 5.17. Mapping results for group 12/52 CH + MTA(W), mapping No. 3. Dotted line shows the approximate margin of the MTA.

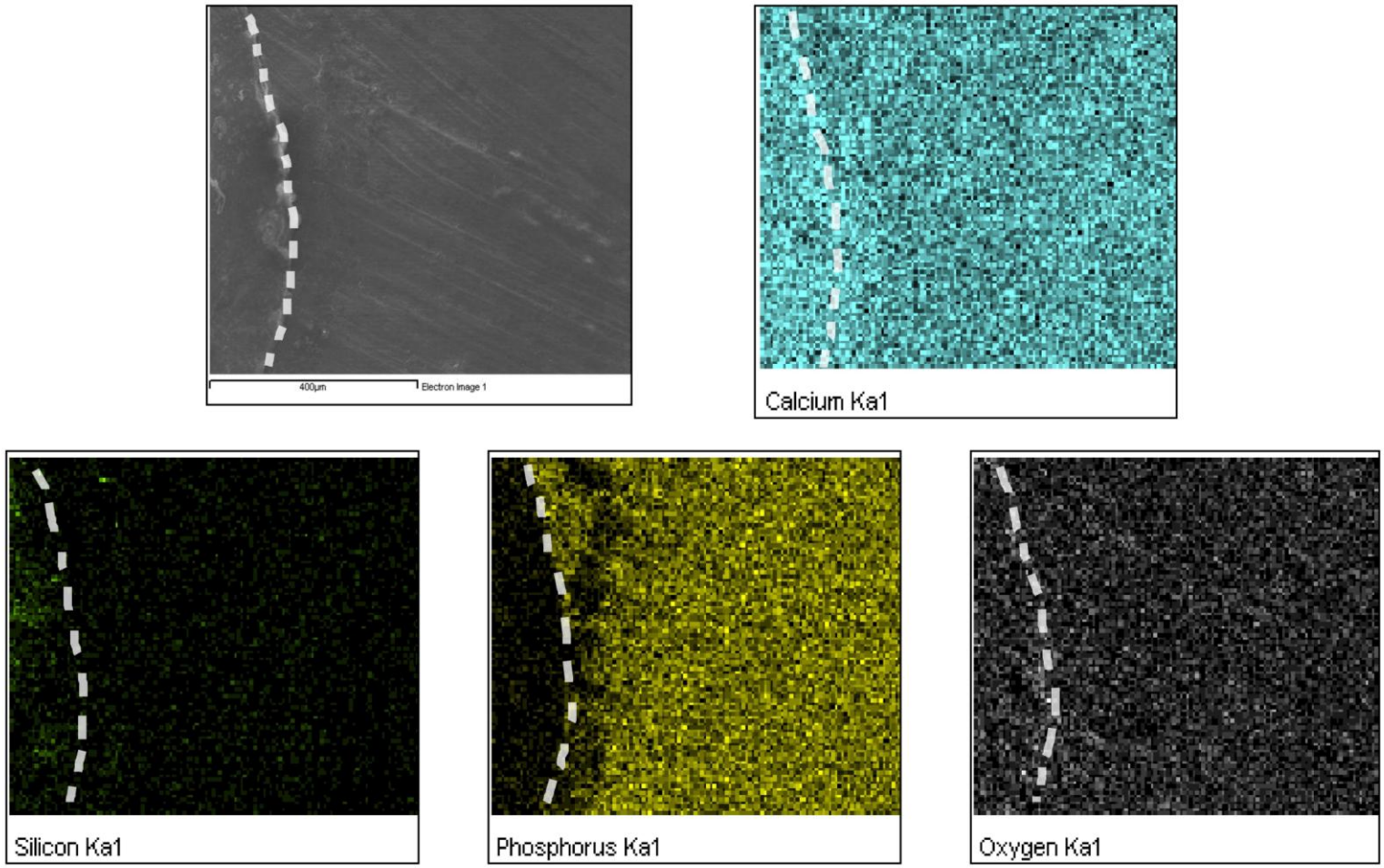


Figure 5.18. Mapping results for group 12/52 CH + MTA(W), mapping No. 4. Dotted line shows the approximate margin of the MTA.

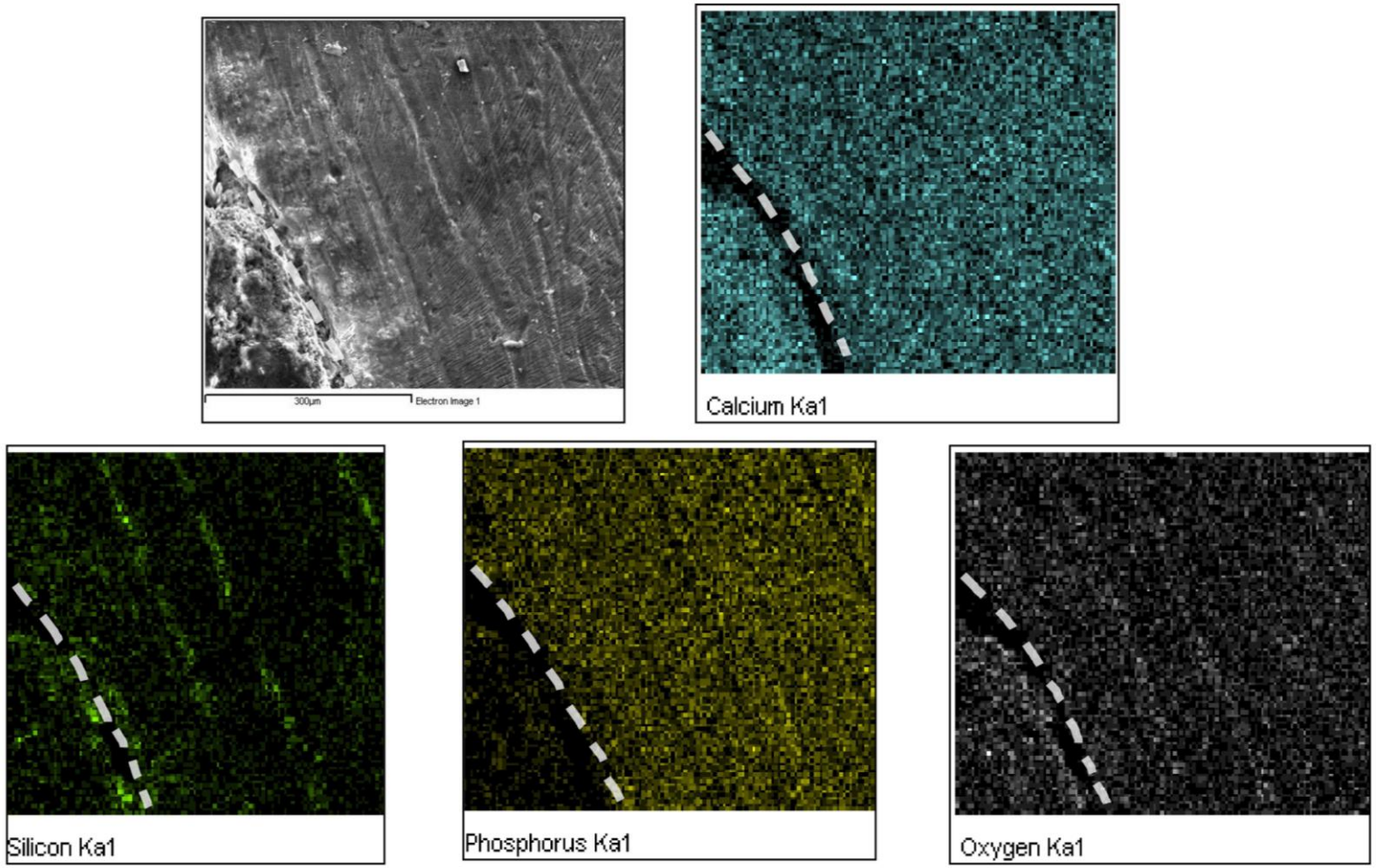


Figure 5.19. Mapping results for group 2/52 CH + MTA(W), mapping No. 1. Dotted line shows the approximate margin of the MTA.

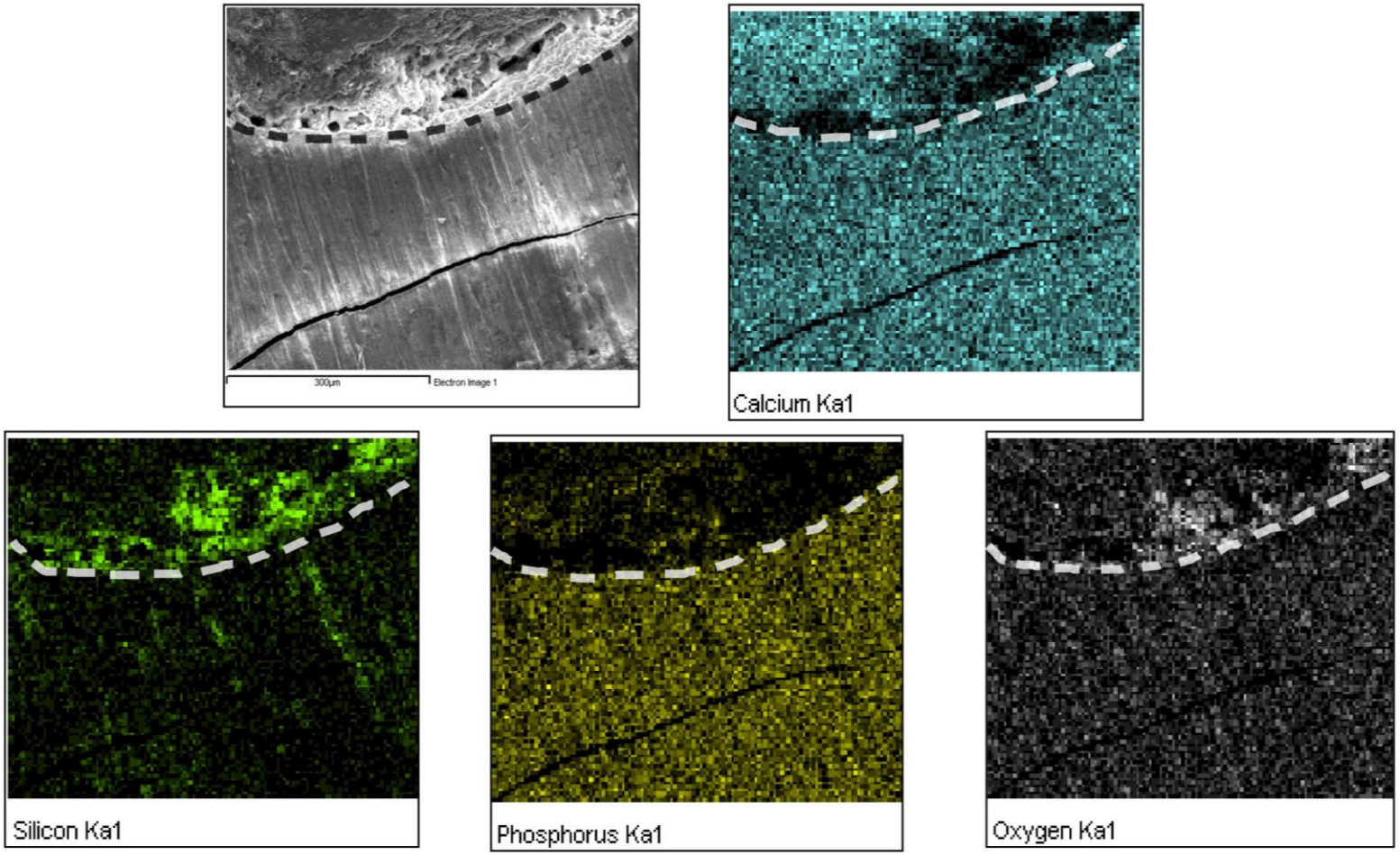


Figure 5.20. Mapping results for group 2/52 CH + MTA(W), Mapping No. 2. Dotted line shows the approximate margin of the MTA.

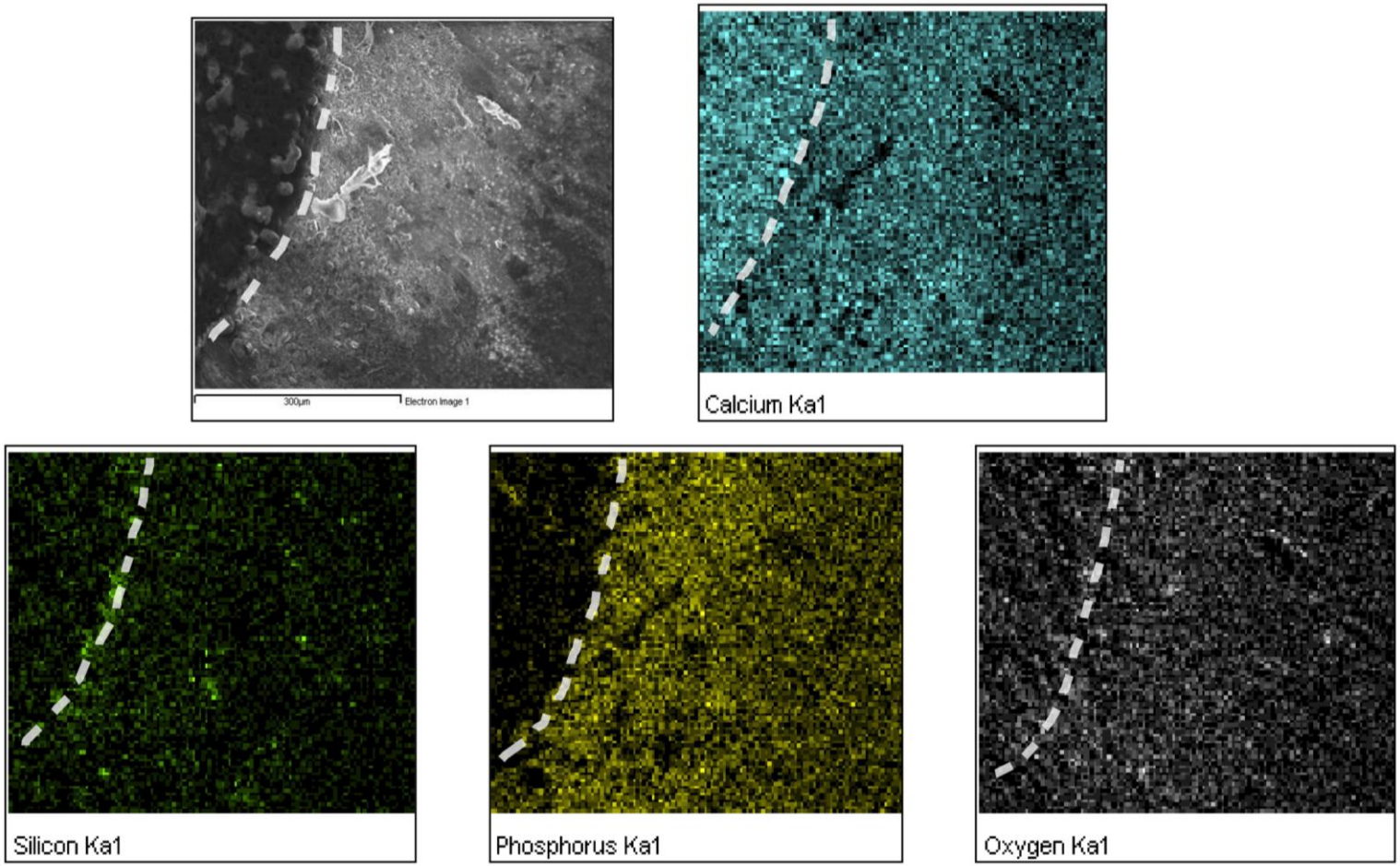


Figure 5.21. Mapping results for group 2/52 CH + MTA(W), mapping No. 3. Dotted line shows the approximate margin of the MTA.

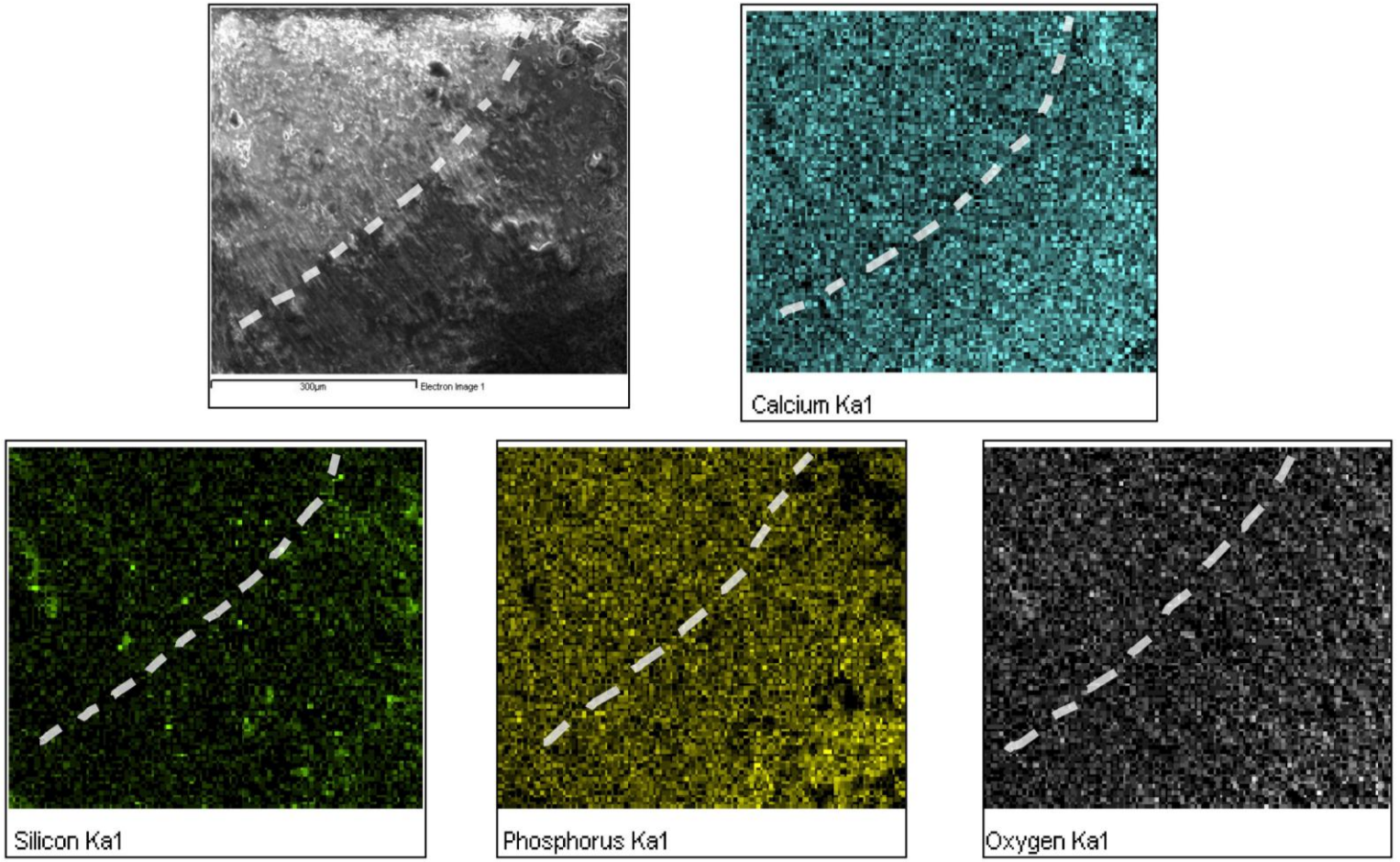


Figure 5.22. Mapping results for group 2/52 CH + MTA(W), mapping No. 4. Dotted line shows the approximate margin of the MTA.

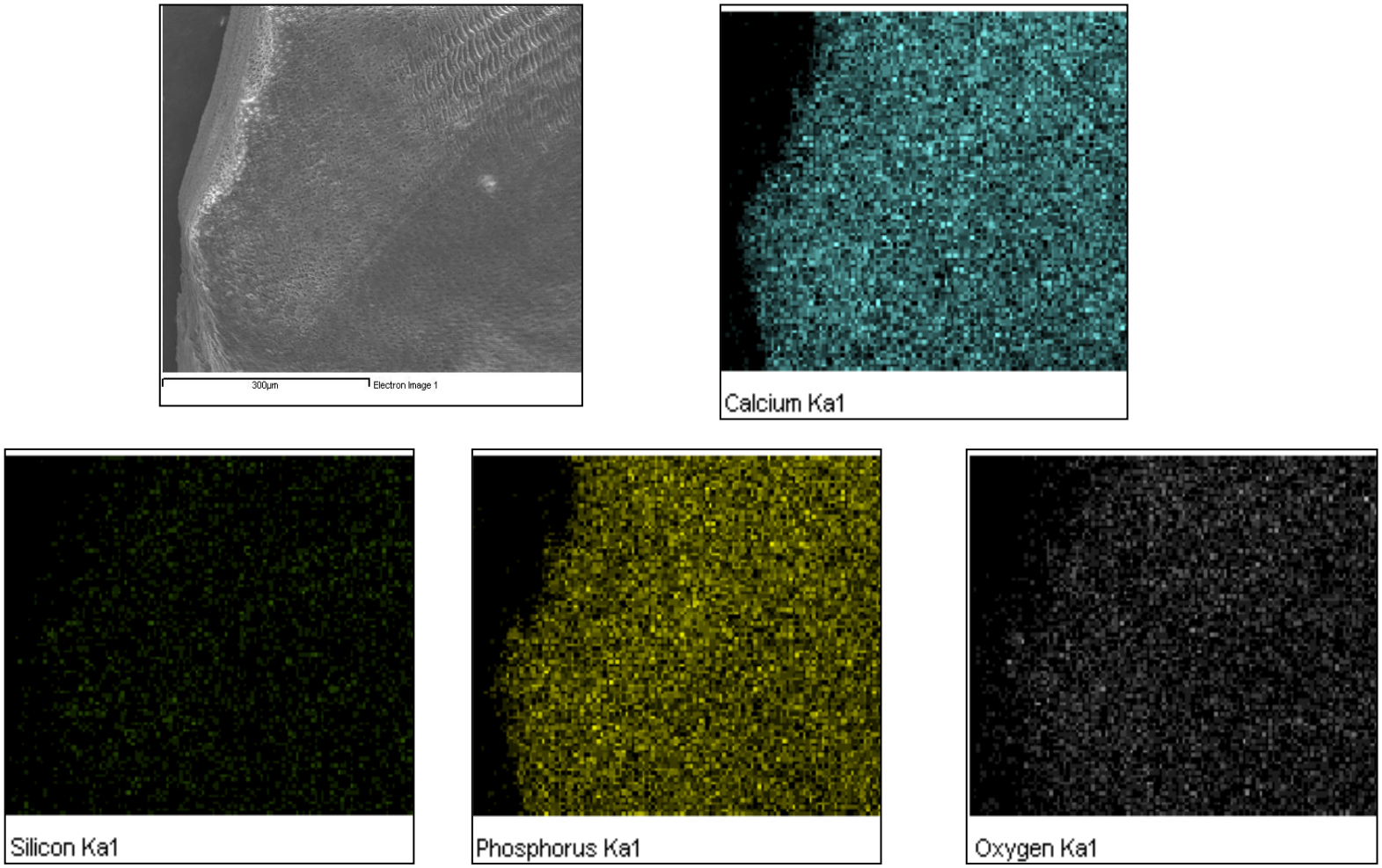


Figure 5.23. Mapping results for group IRRIGATION ONLY, mapping No. 1.

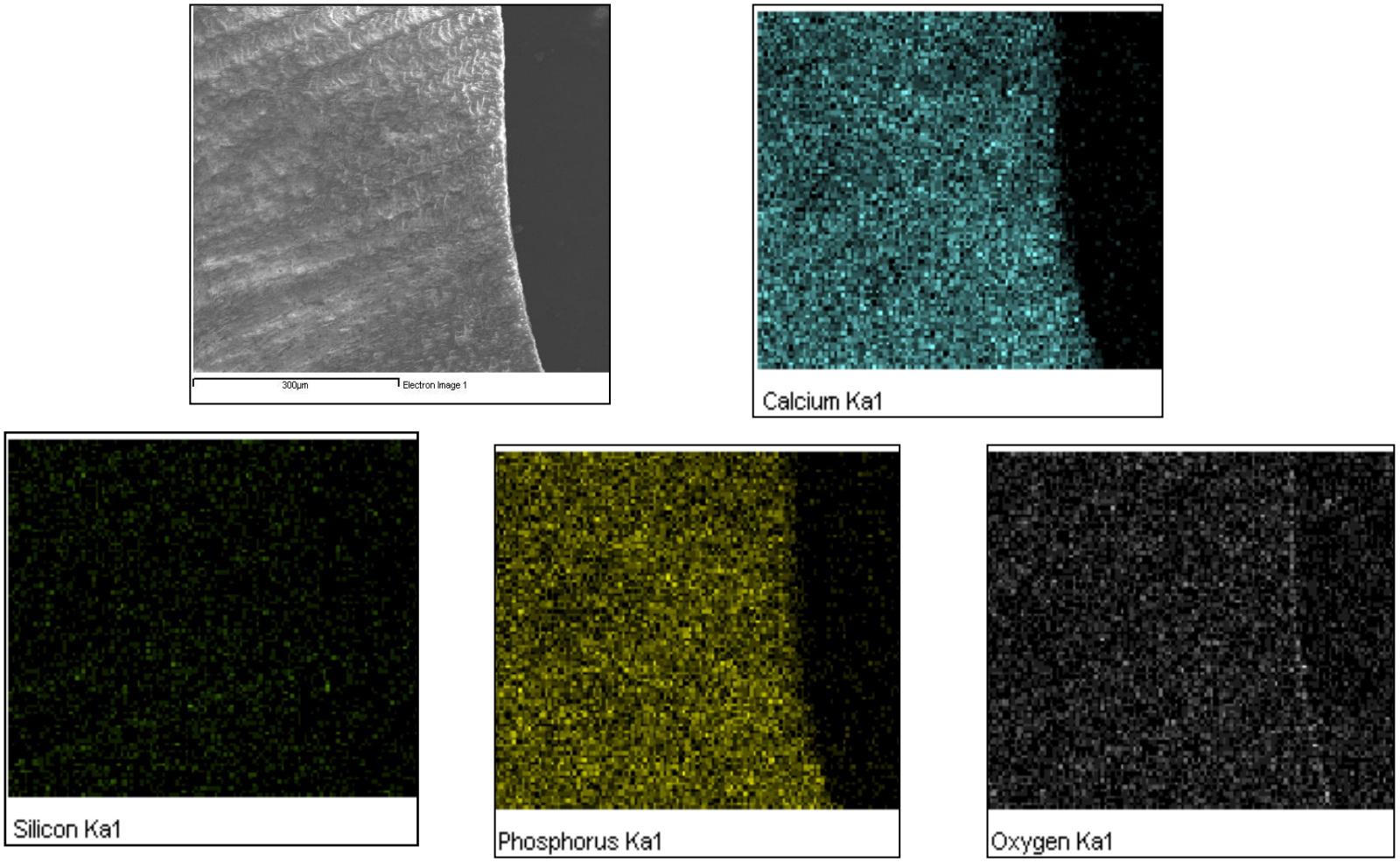


Figure 5.24. Mapping results for group IRRIGATION ONLY, mapping No. 2.

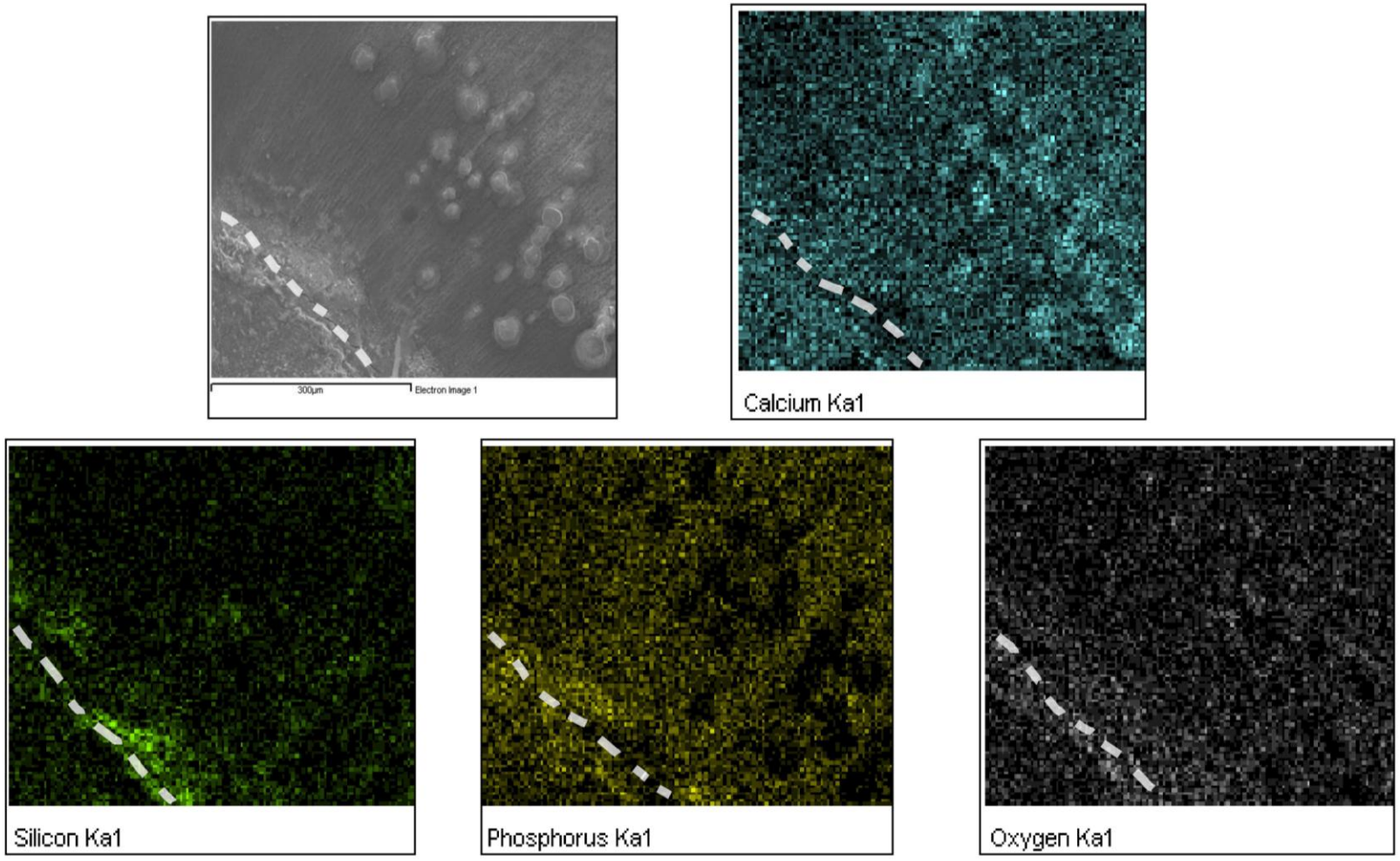


Figure 5.25. Mapping results for group 12/52 CH + MTA(PBS), mapping No. 1. Dotted line shows the approximate margin of the MTA.

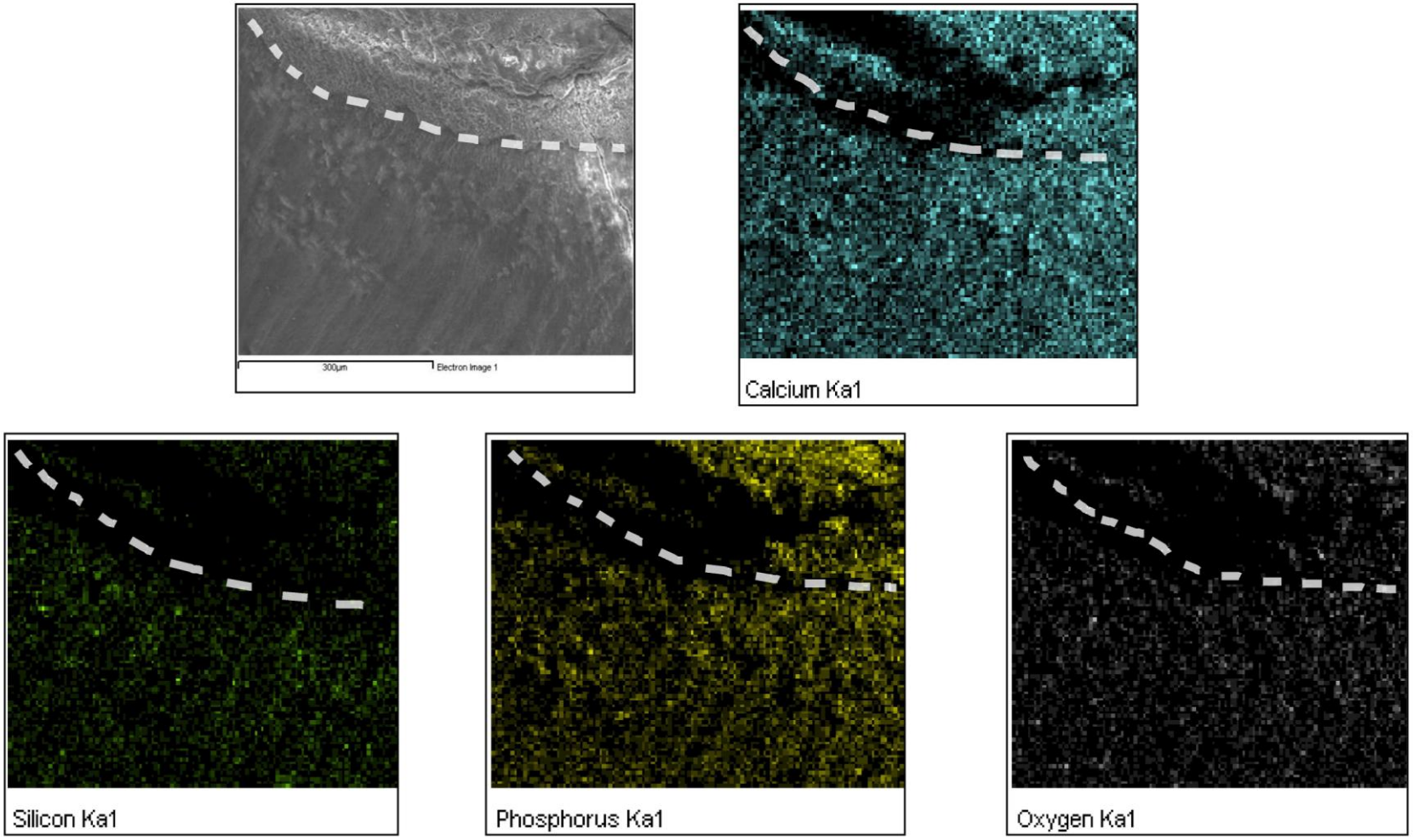


Figure 5.26. Mapping results for group 12/52 CH + MTA(PBS), mapping No. 2. Dotted line shows the approximate margin of the MTA.

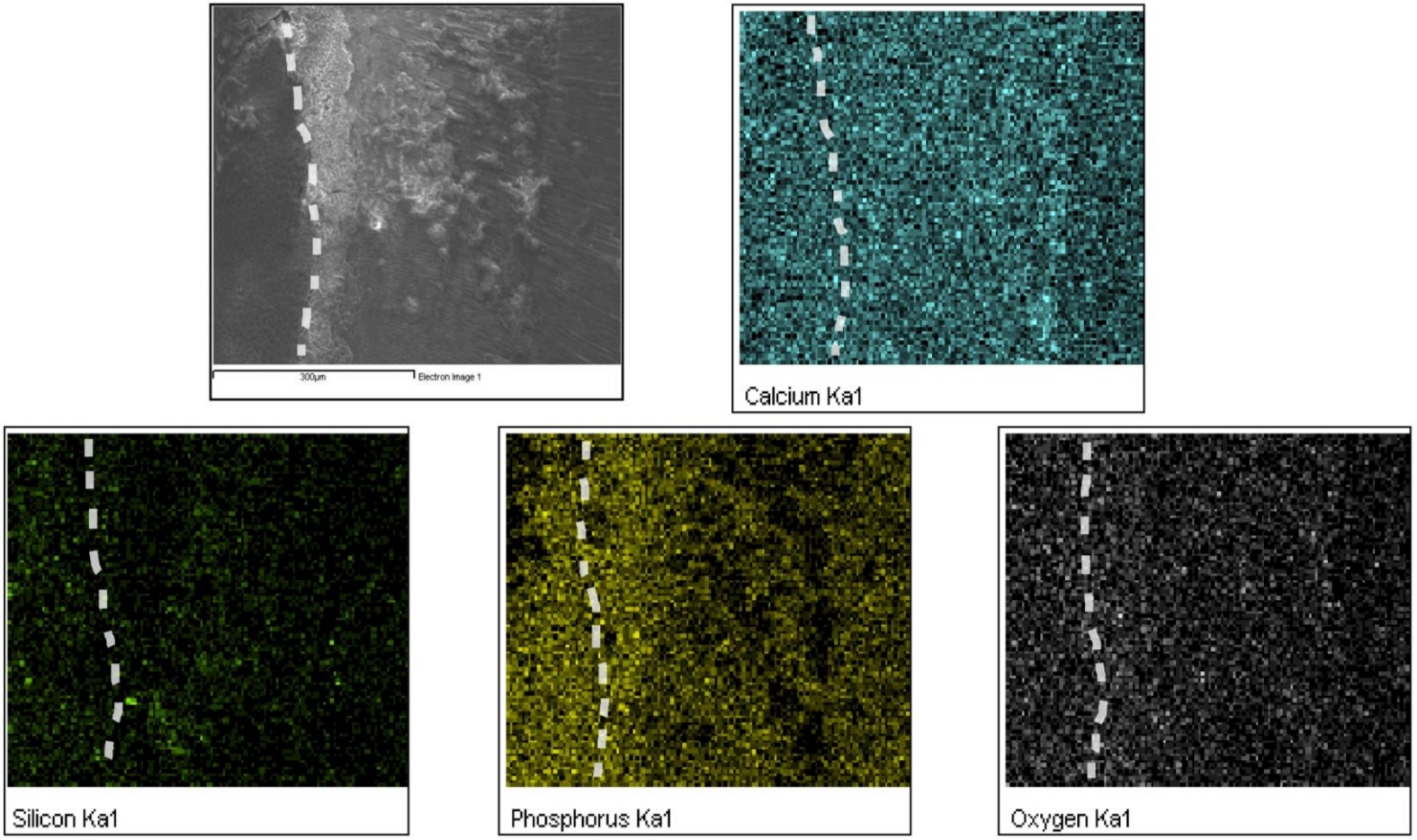


Figure 5.27. Mapping results for group 12/52 CH + MTA(PBS), mapping No. 3. Dotted line shows the approximate margin of the MTA.

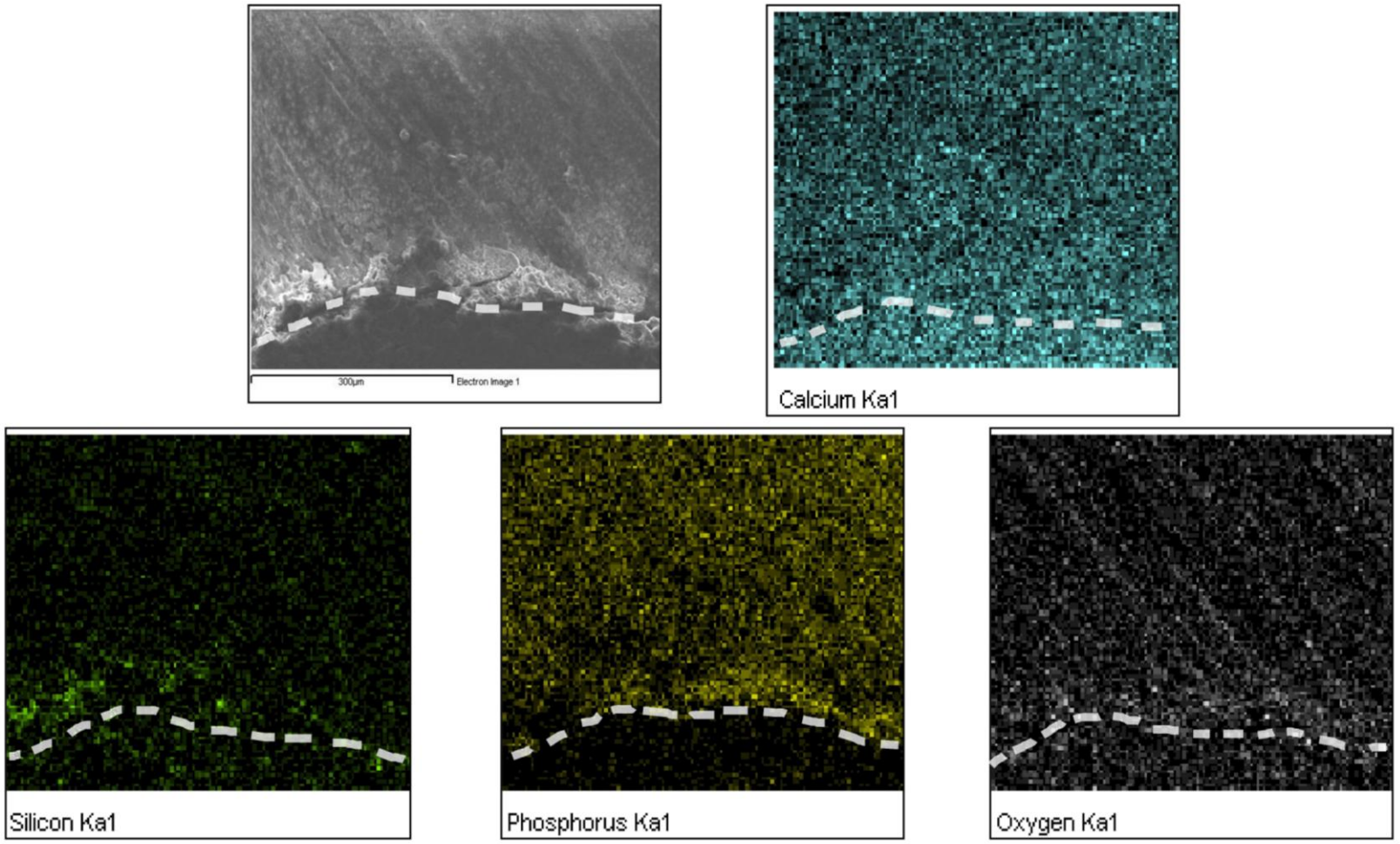


Figure 5.28. Mapping results for group 12/52 CH + MTA(PBS), mapping No. 4. Dotted line shows the approximate margin of the MTA.

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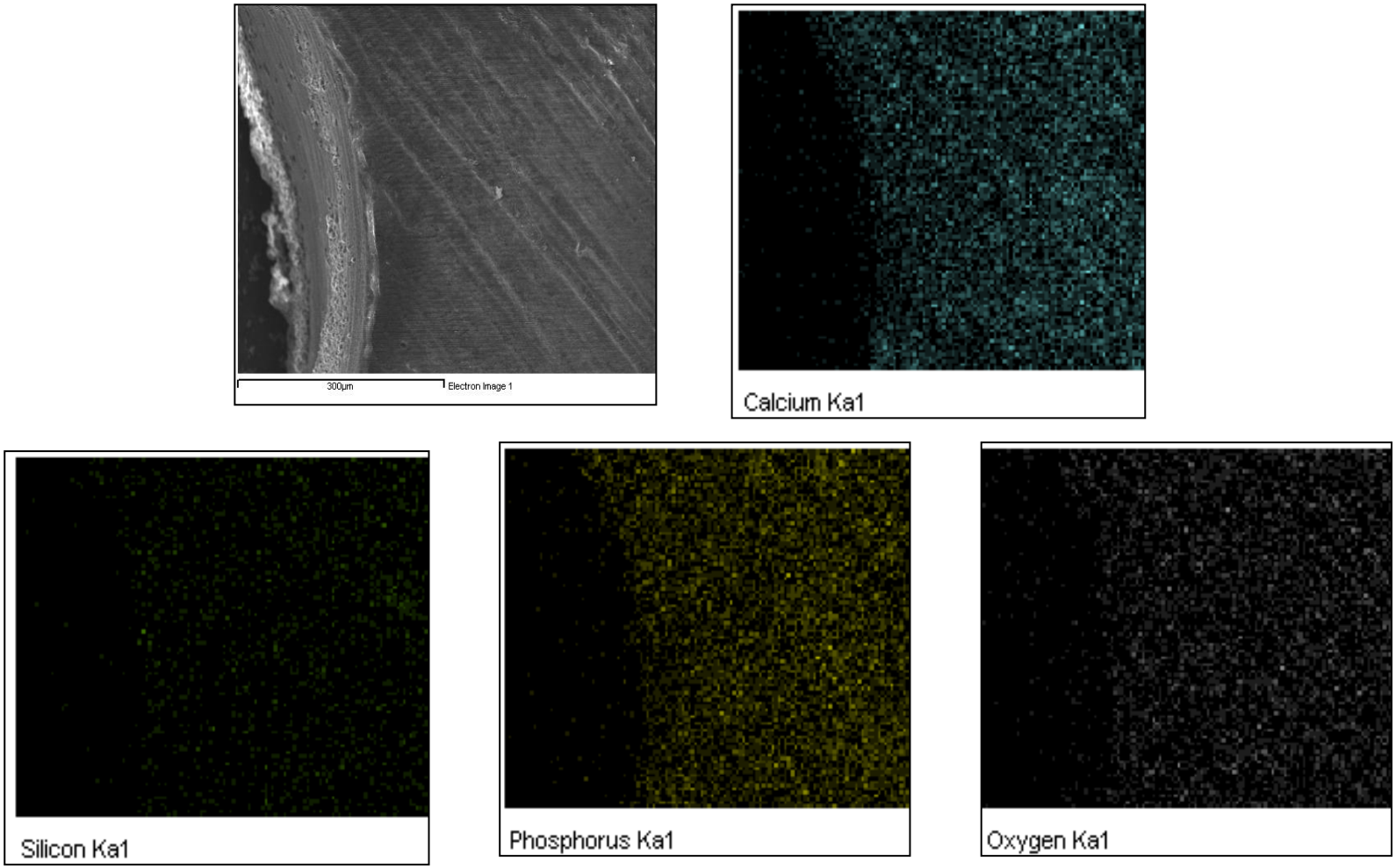

Figure 5.29. Mapping results for group 2/52 CH, mapping No. 1.

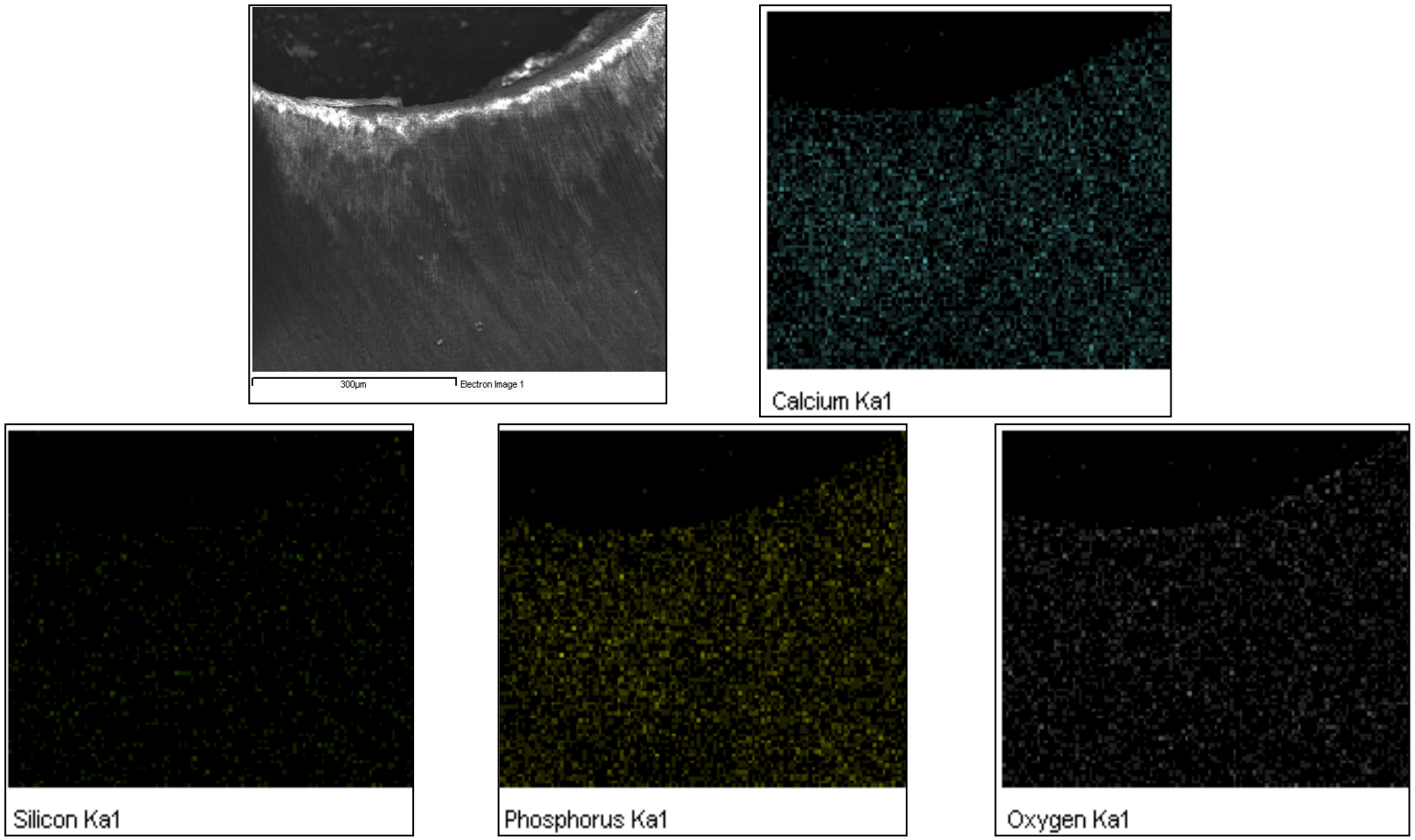

Figure 5.30. Mapping results for group 2/52 CH, mapping No. 2.

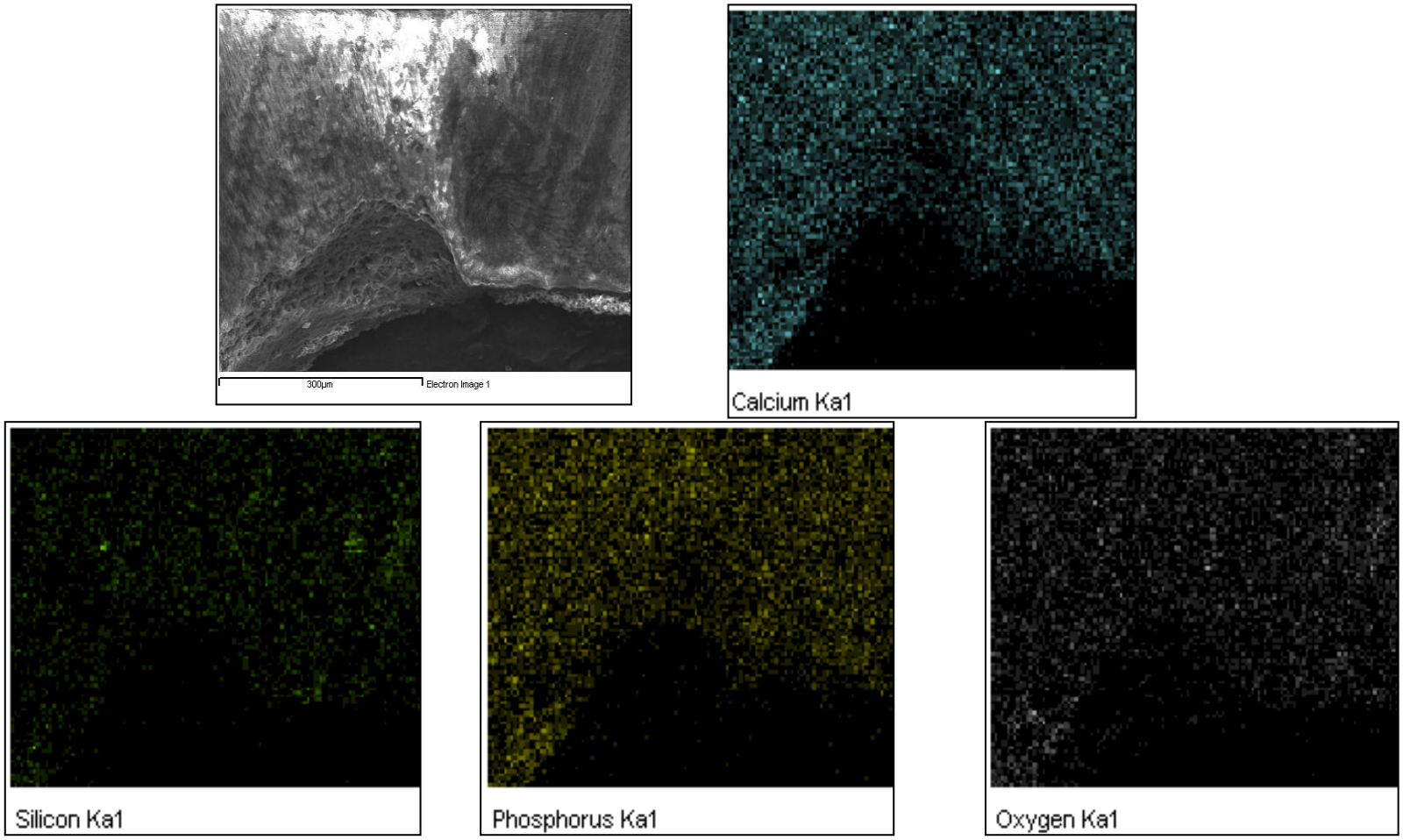

Figure 5.31. Mapping results for group 2/52 CH, mapping No. 3.

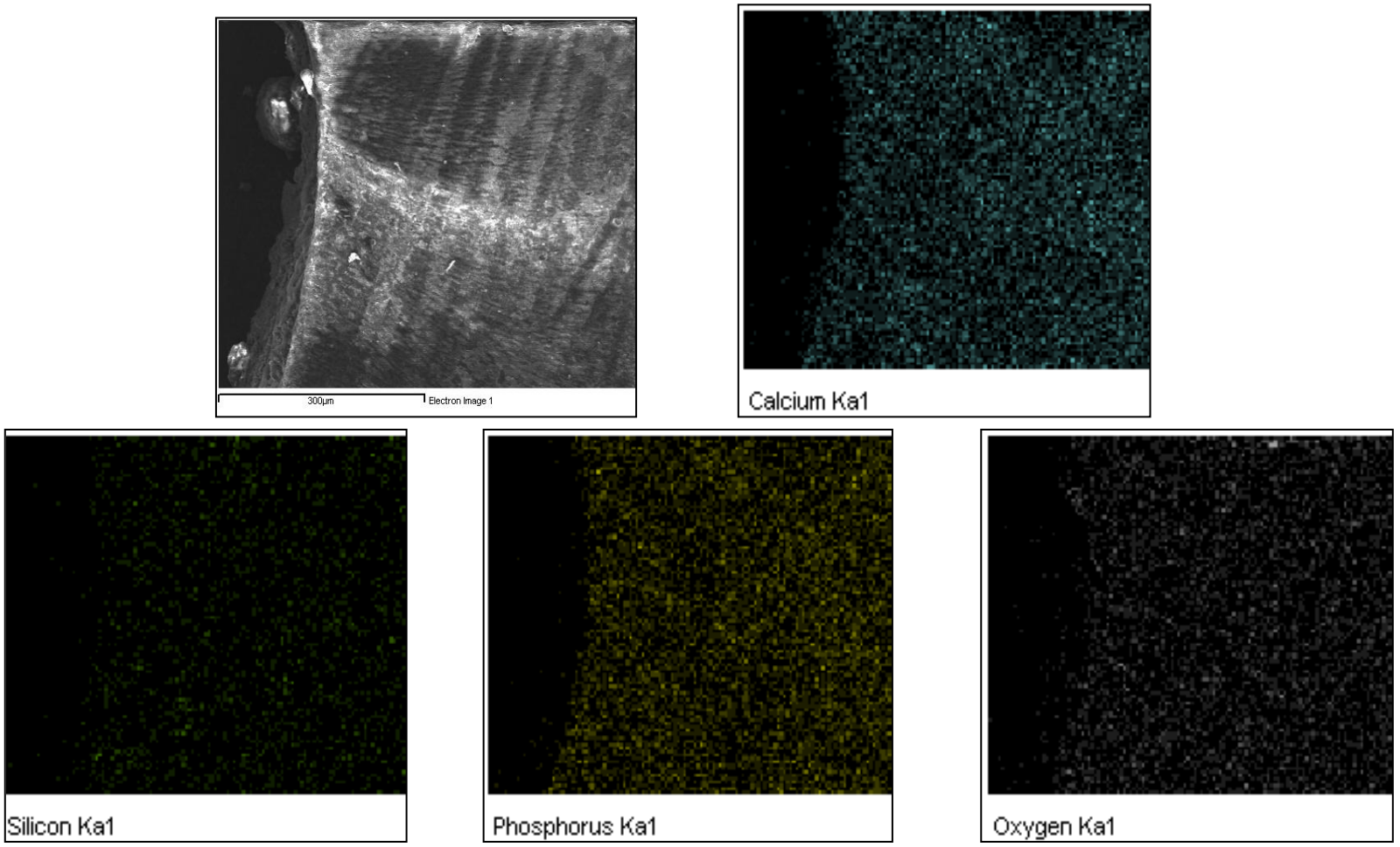

Figure 5.32. Mapping results for group 2/52 CH, mapping No. 4.

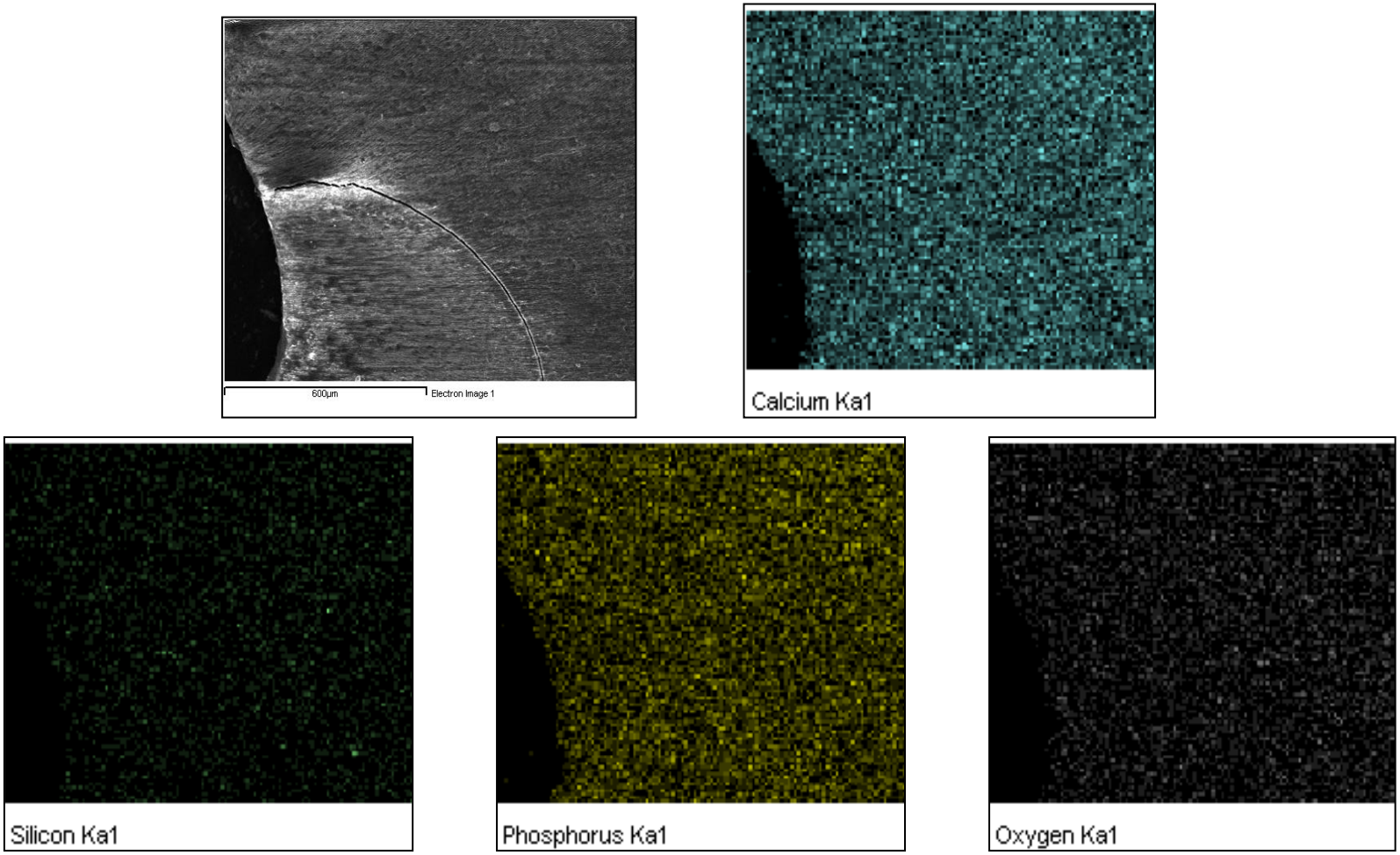

Figure 5.33. Mapping results for group 12/52 CH, mapping No. 1.

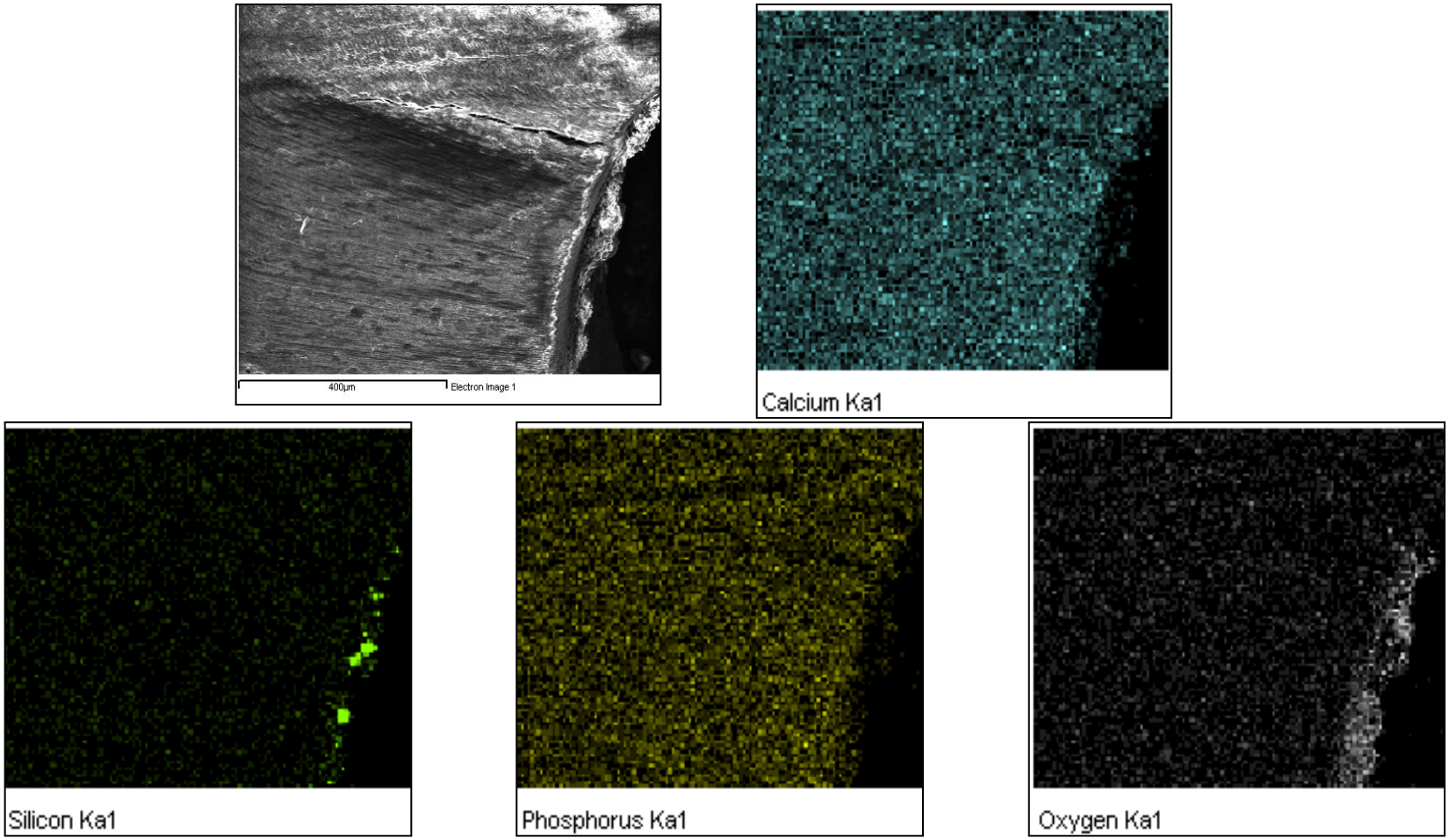

Figure 5.34. Mapping results for group 12/52 CH, mapping No. 2.

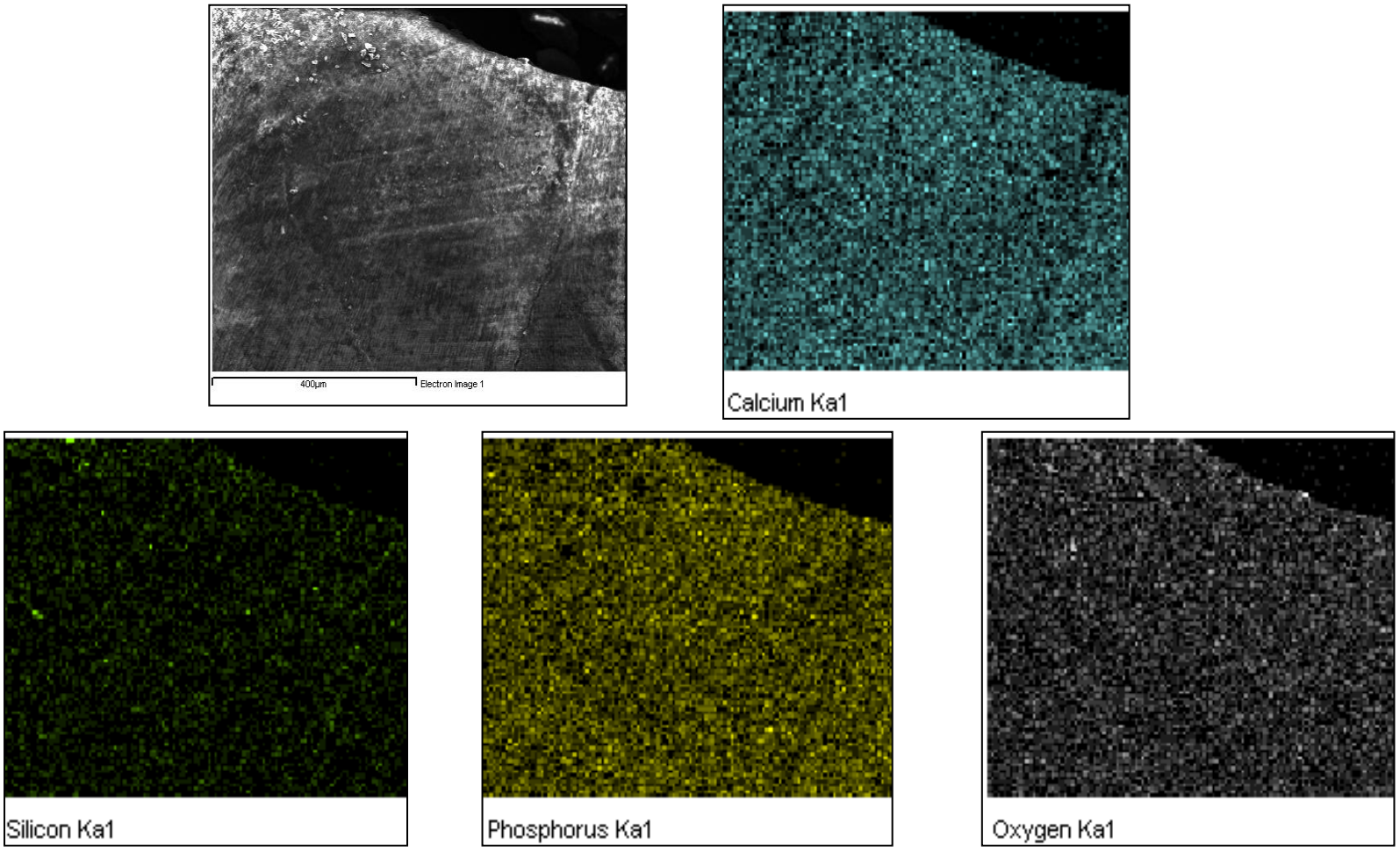

Figure 5.35. Mapping results for group 12/52 CH, mapping No. 3.

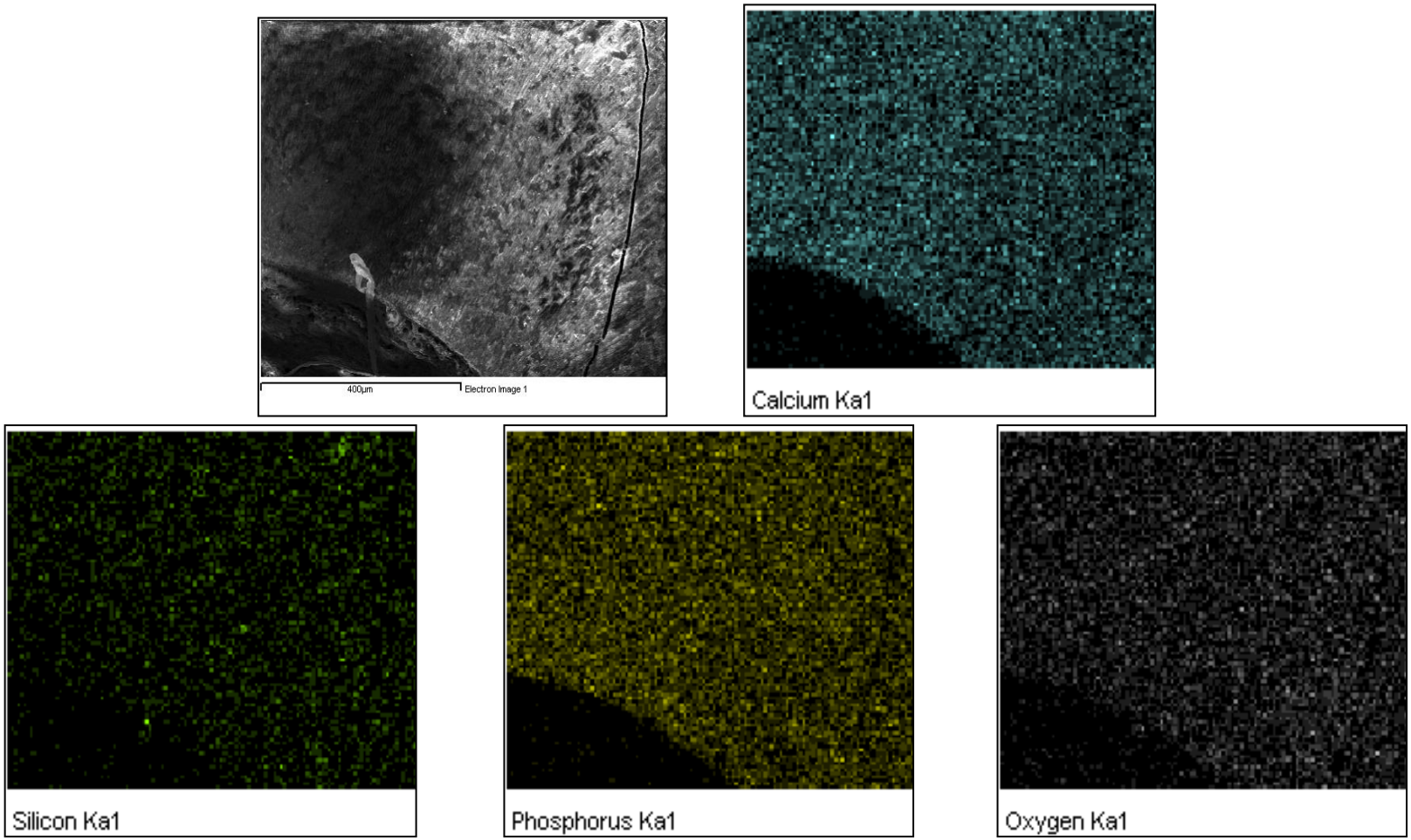

Figure 5.36. Mapping results for group 12/52 CH, mapping No. 4.

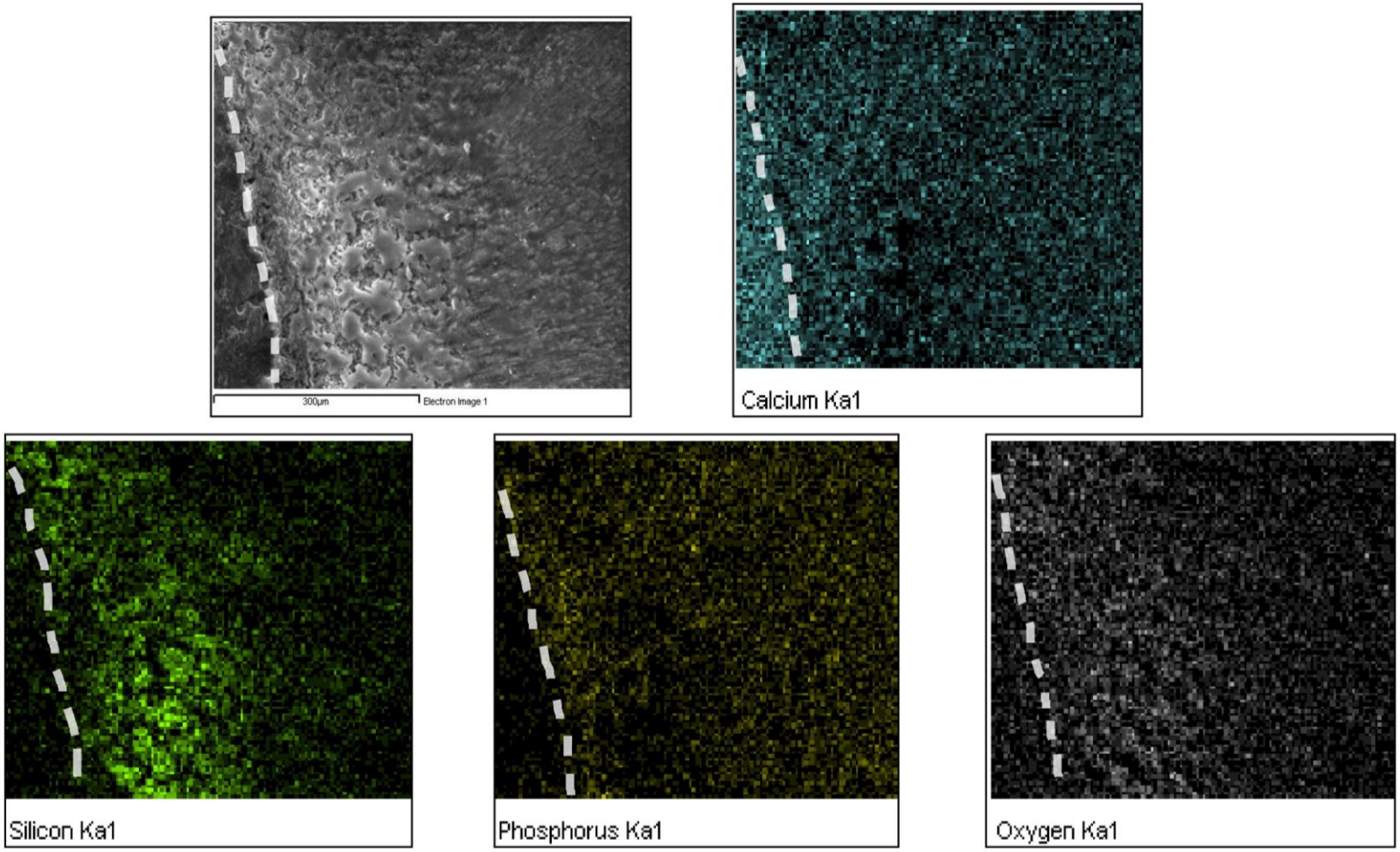

Figure 5.37. Mapping results for group 2/52 CH + MTA(PBS), mapping No. 1. Dotted line shows the approximate margin of the MTA.

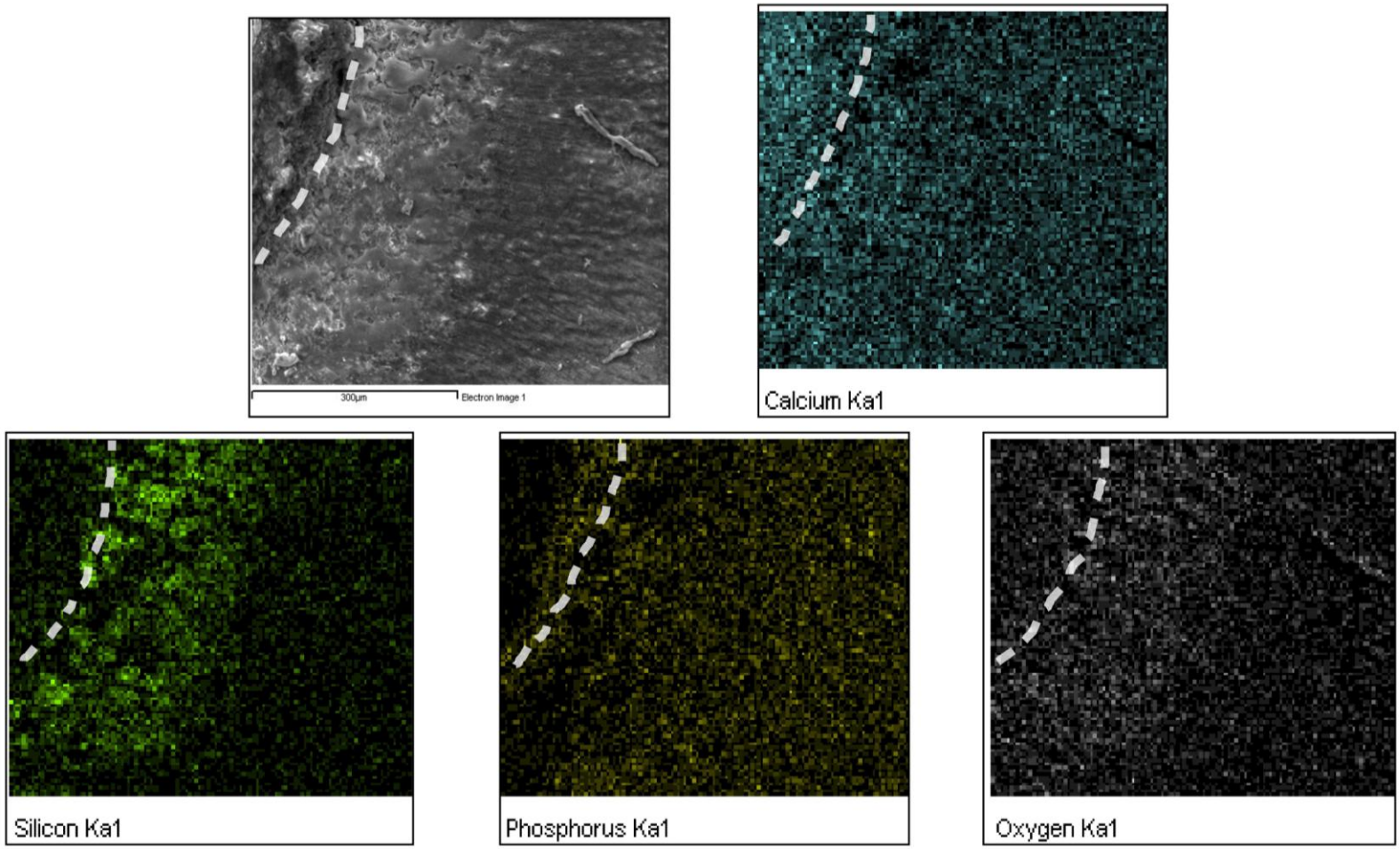

Figure 5.38. Mapping results for group 2/52 CH + MTA(PBS), mapping No. 2. Dotted line shows the approximate margin of the MTA.

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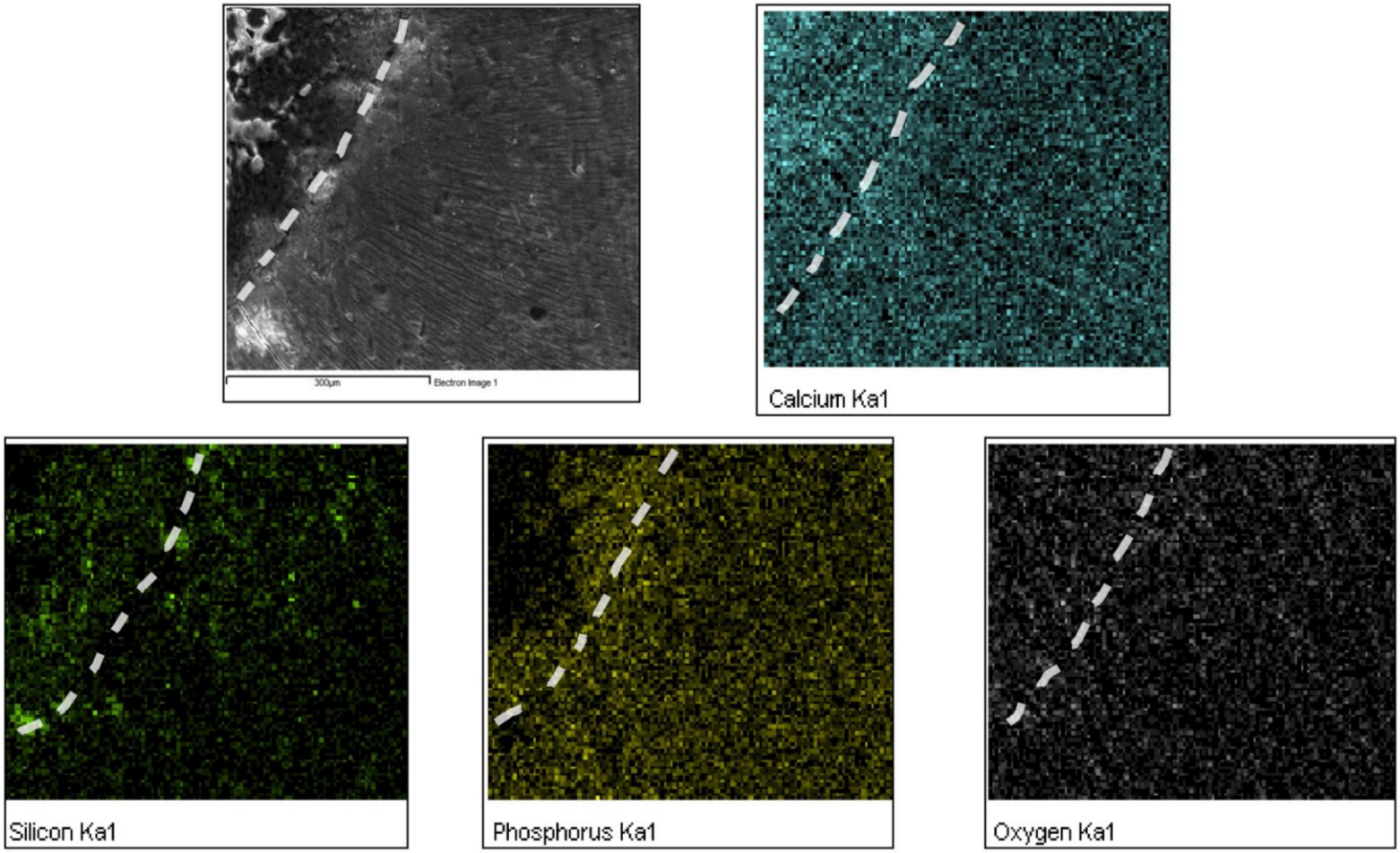

Figure 5.39. Mapping results for group 2/52 CH + MTA(PBS), mapping No. 3. Dotted line shows the approximate margin of the MTA.

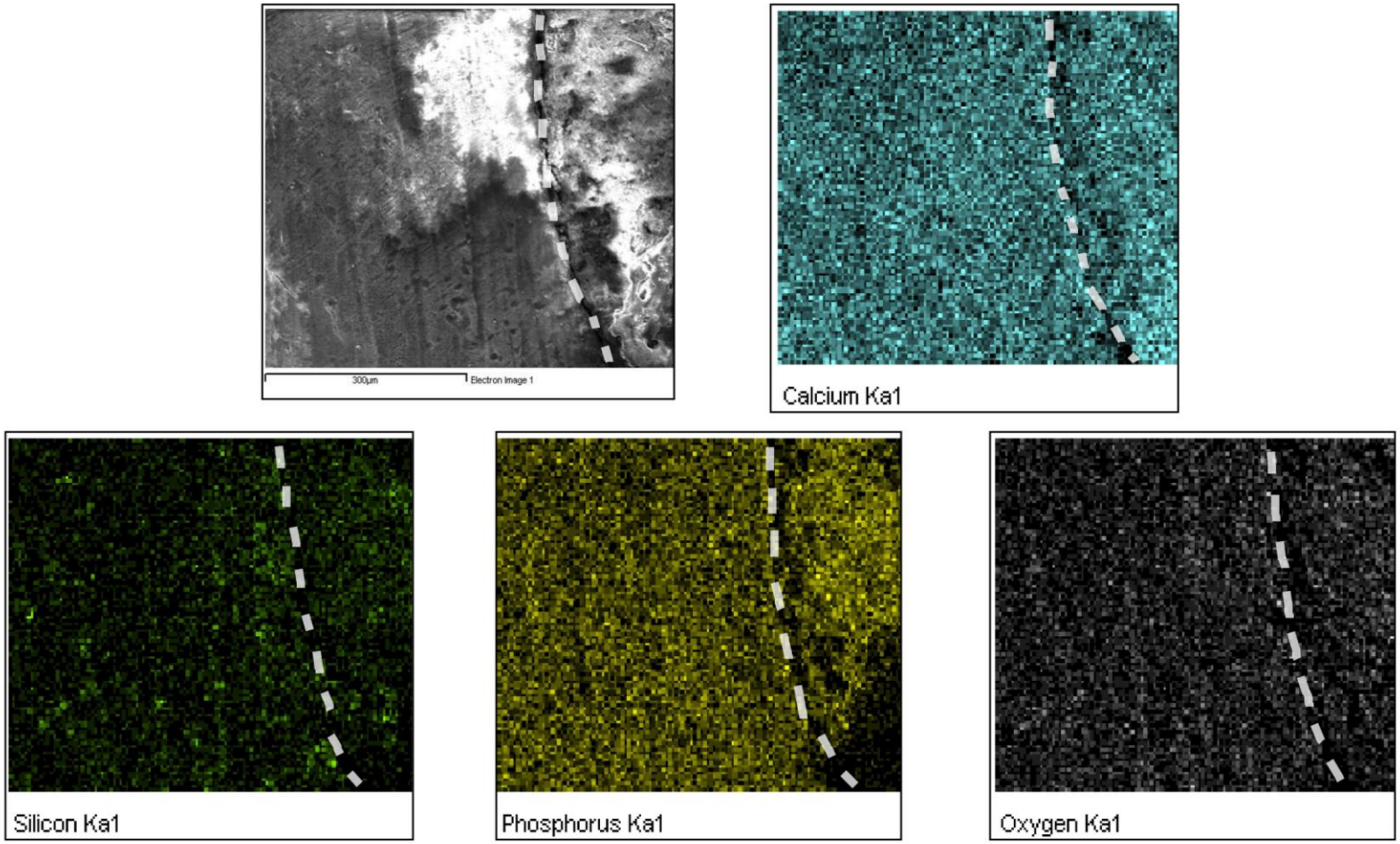

Figure 5.40. Mapping results for group 2/52 CH + MTA(PBS), mapping No. 4. Dotted line shows the approximate margin of the MTA.

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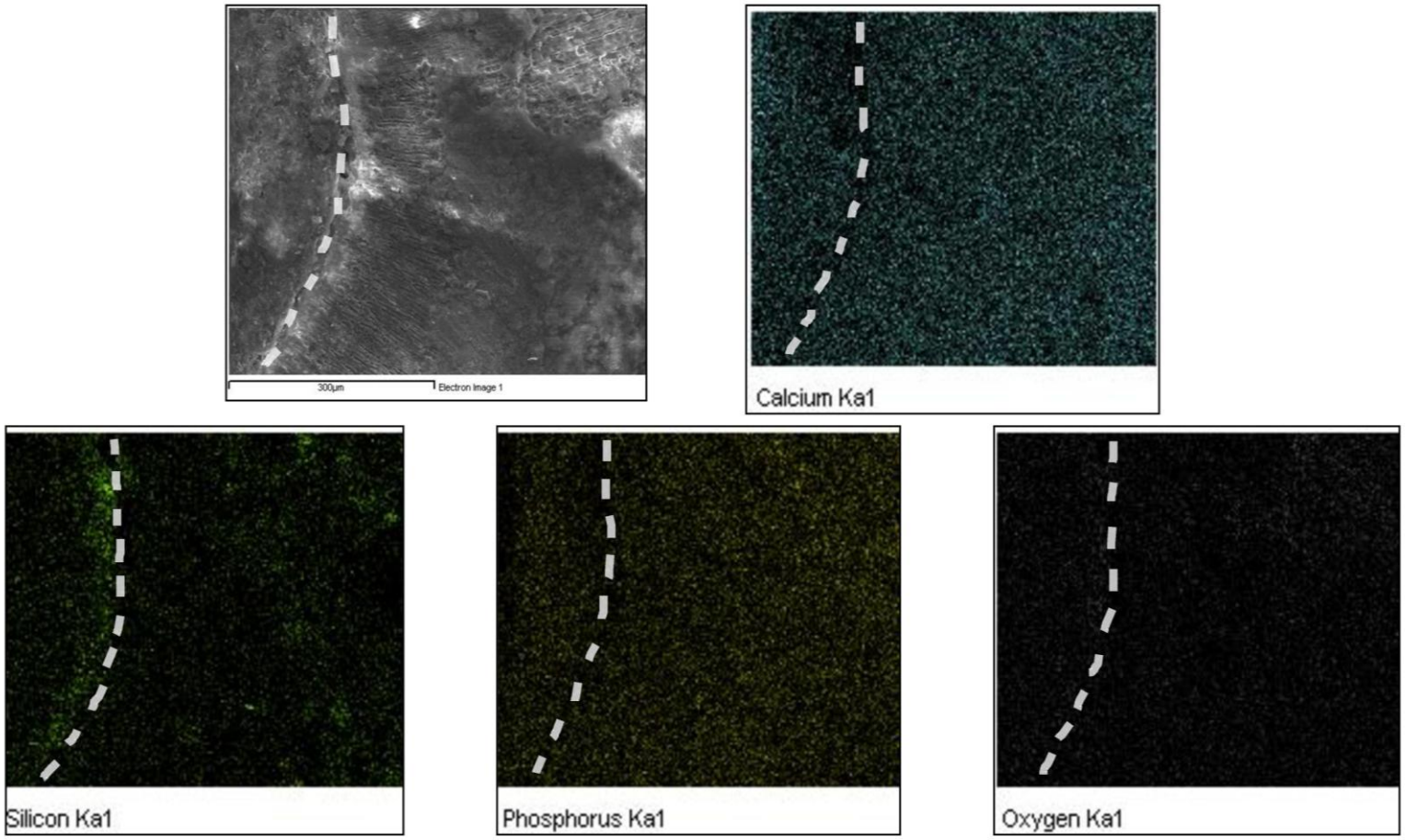

Figure 5.41. Mapping results for group MTA(PBS), mapping No. 1. Dotted line shows the approximate margin of the MTA.

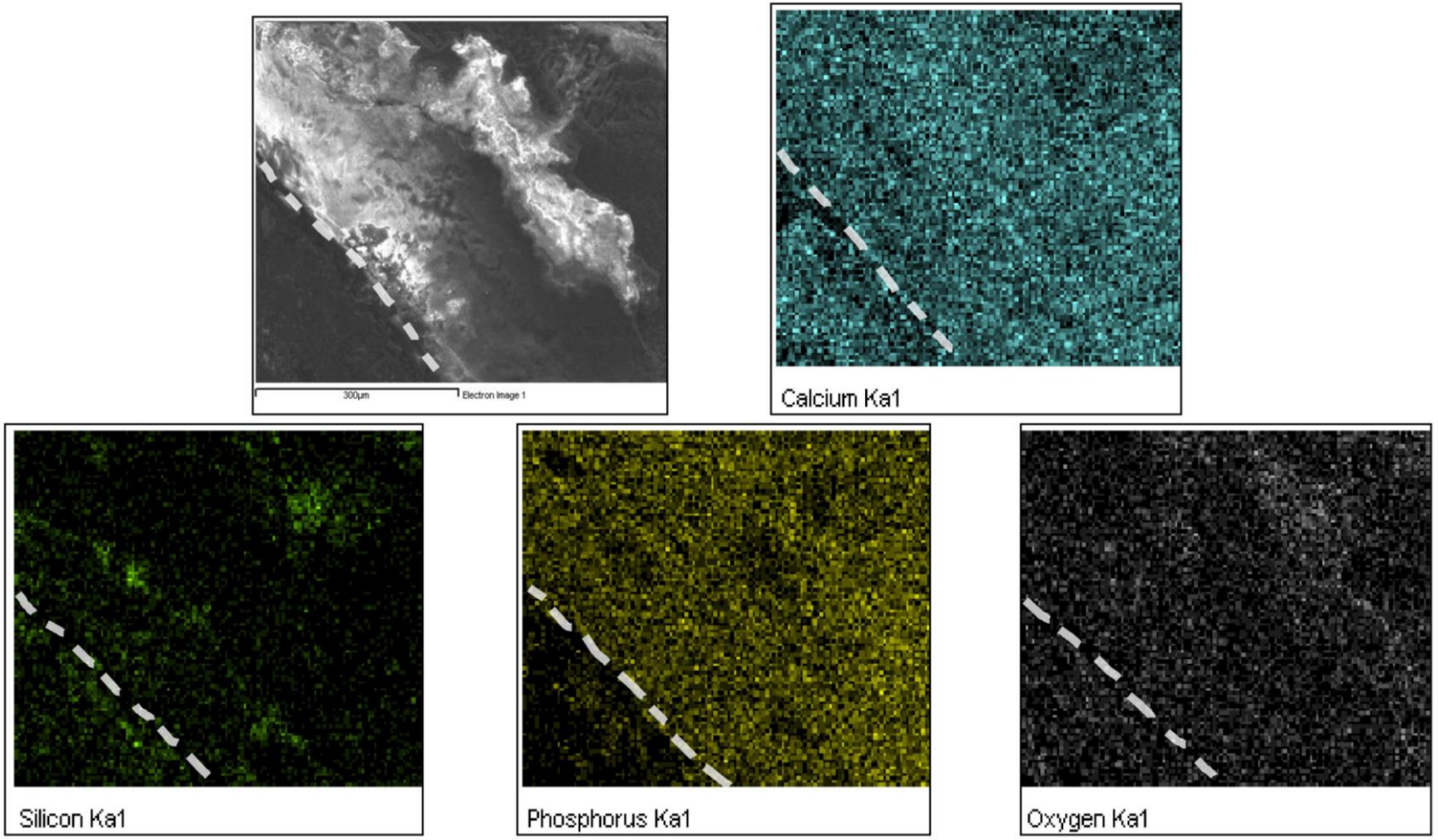

Figure 5.42. Mapping results for group MTA(PBS), mapping No. 2. Dotted line shows the approximate margin of the MTA.

.

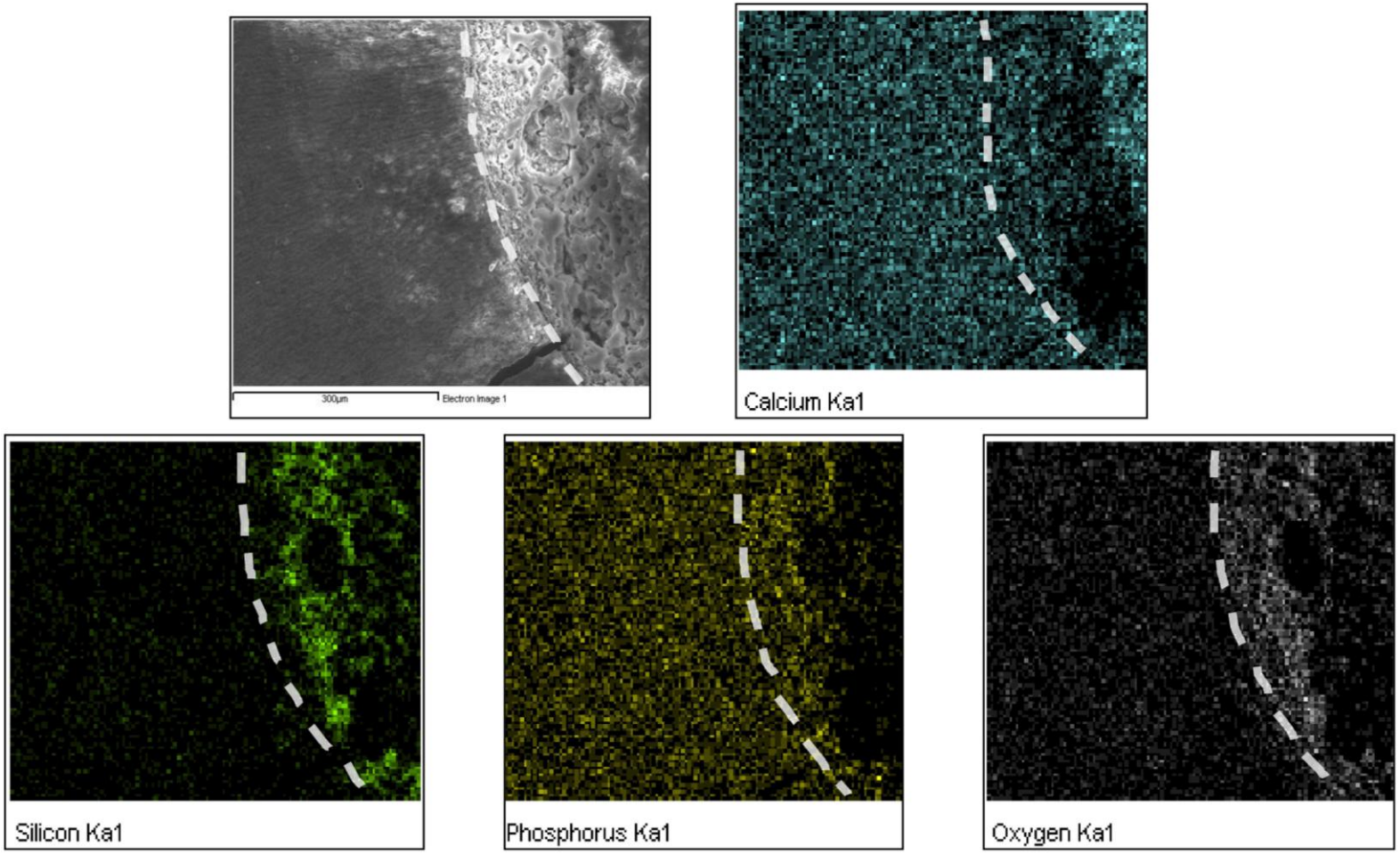

Figure 5.43. Mapping results for group MTA(PBS), mapping No. 3. Dotted line shows the approximate margin of the MTA.

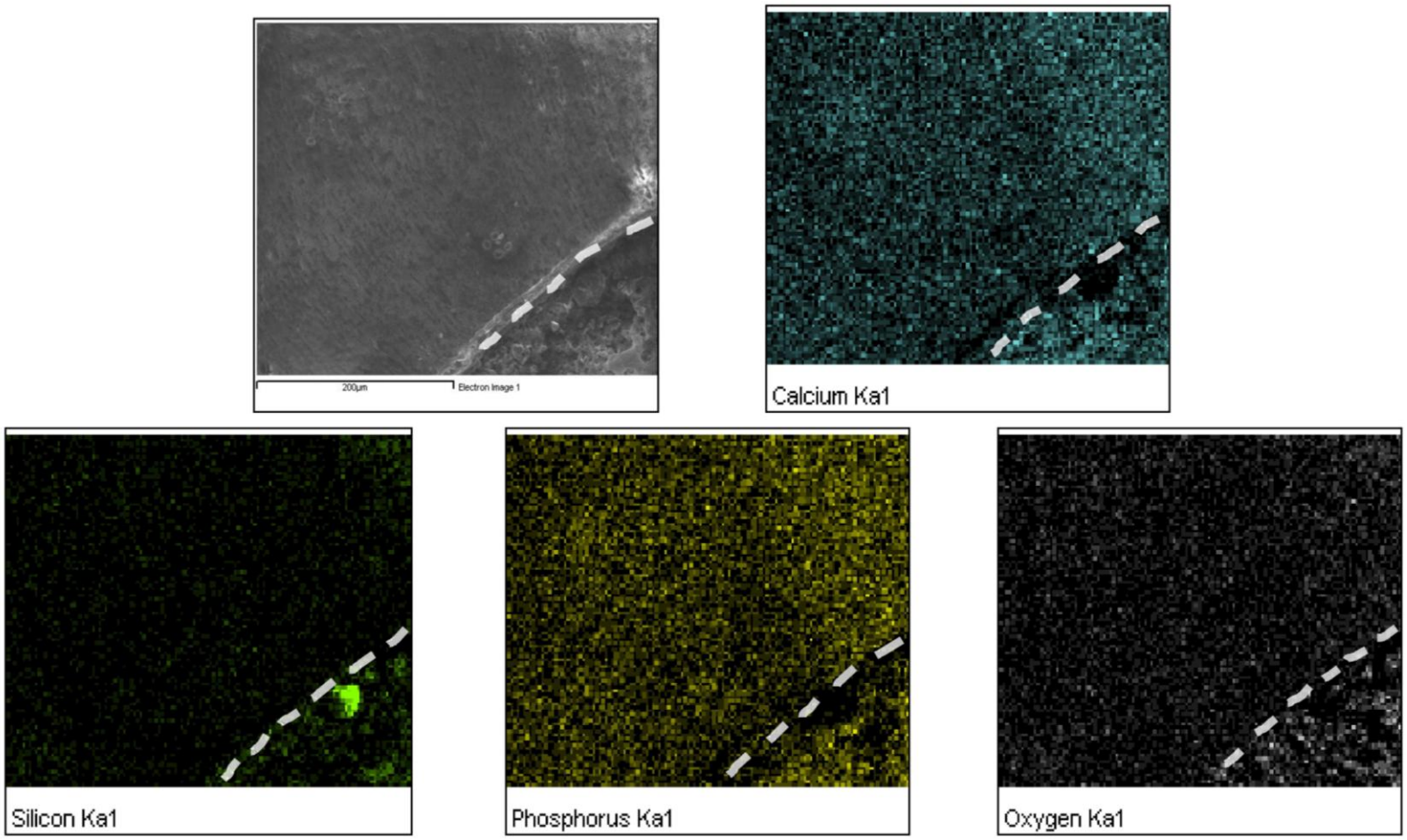

Figure 5.44. Mapping results for group MTA(PBS), mapping No. 4. Dotted line shows the approximate margin of the MTA.

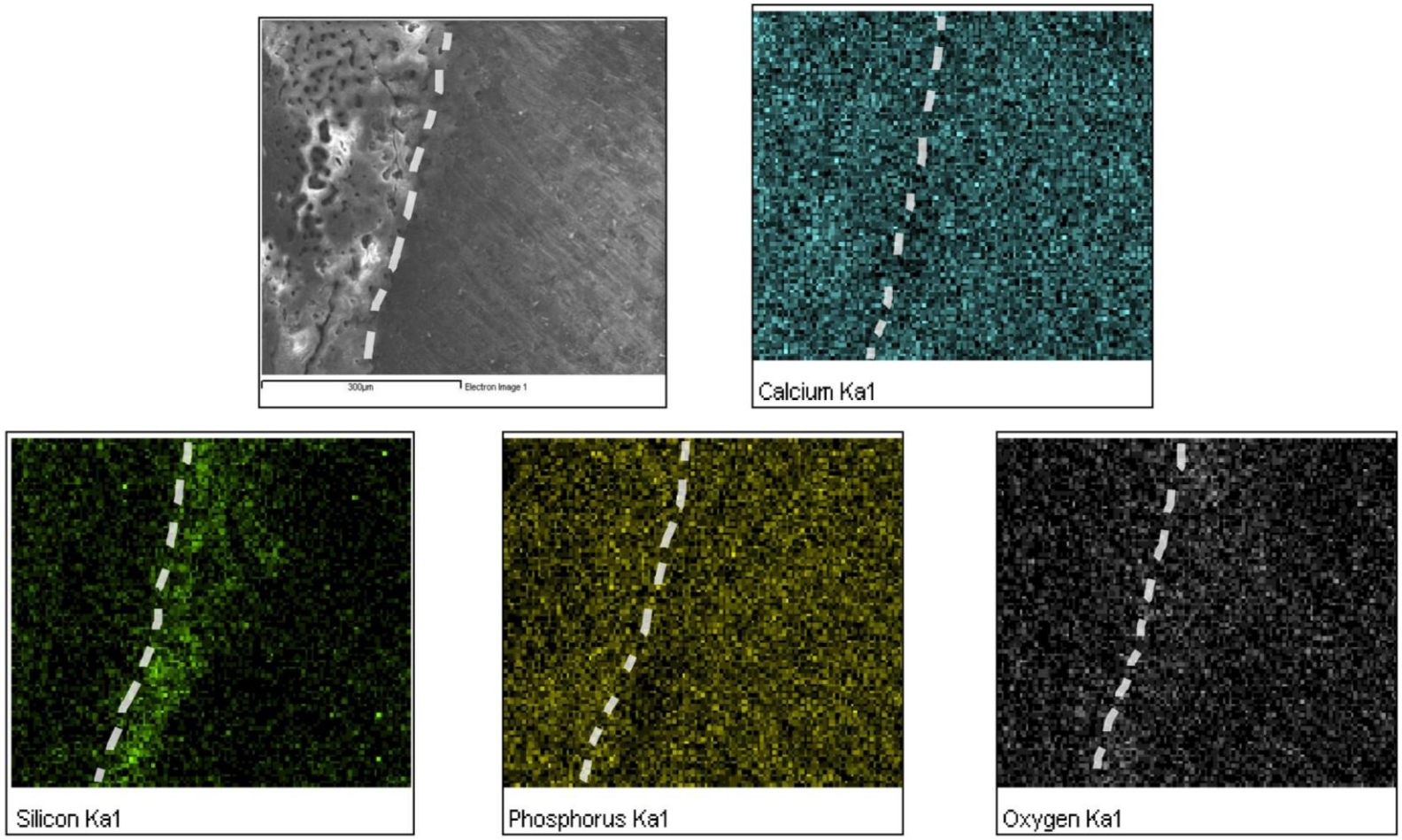

Figure 5.45. Mapping results for group MTA(W), mapping No. 1. Dotted line shows the approximate margin of the MTA.

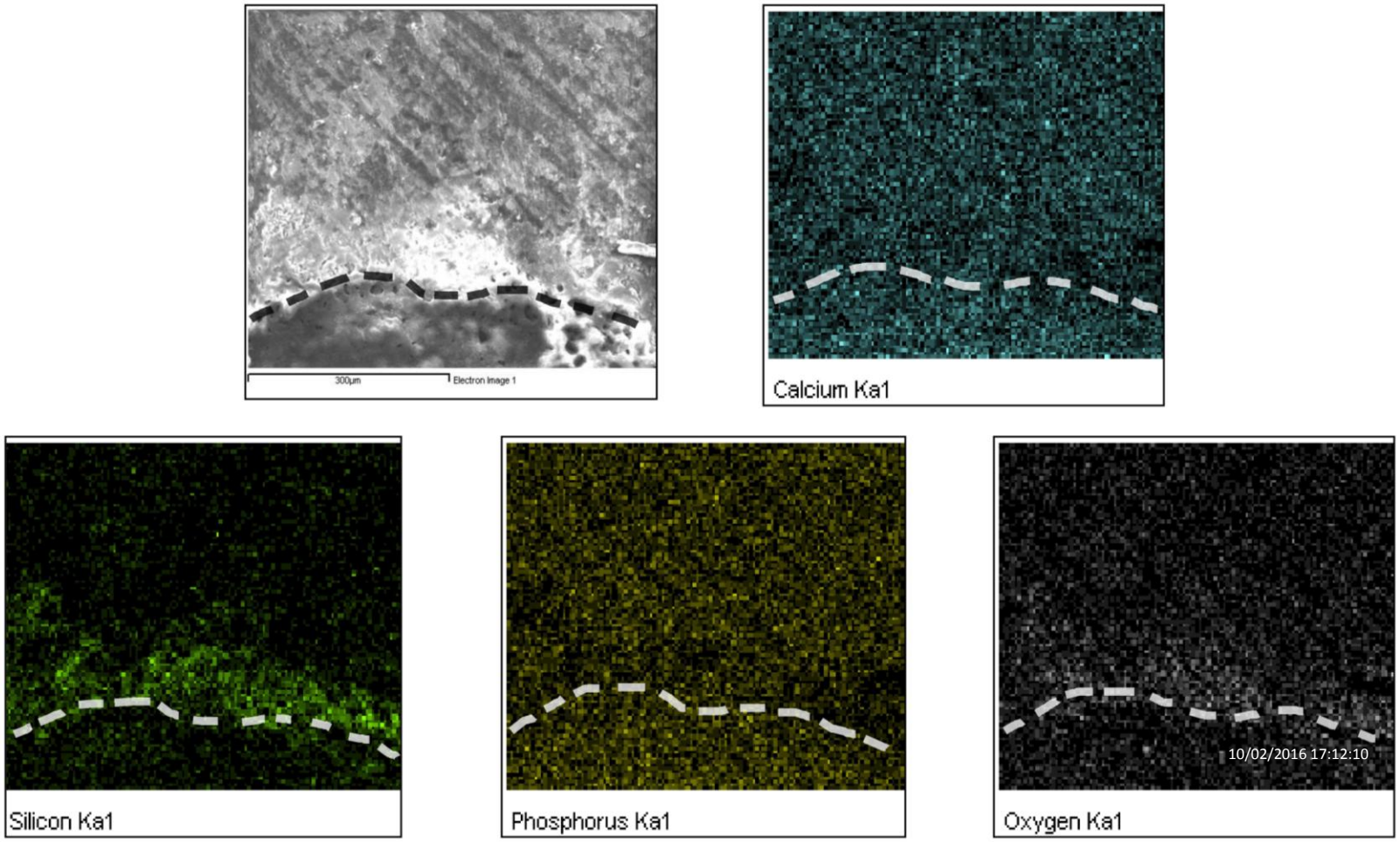

Figure 5.46. Mapping results for group MTA(W), mapping No. 2. Dotted line shows the approximate margin of the MTA.

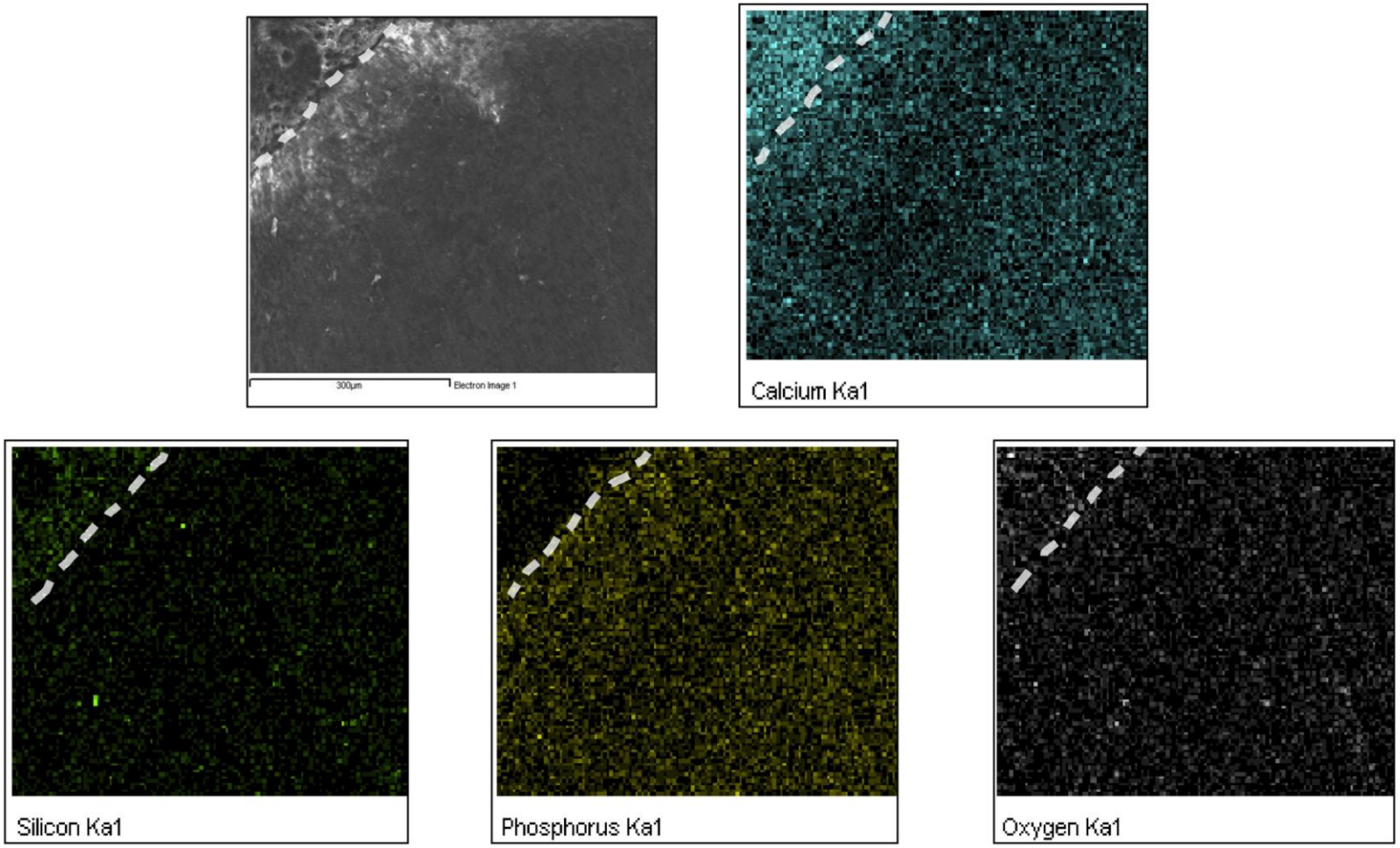

Figure 5.47. Mapping results for group MTA(W), mapping No. 3. Dotted line shows the approximate margin of the MTA.

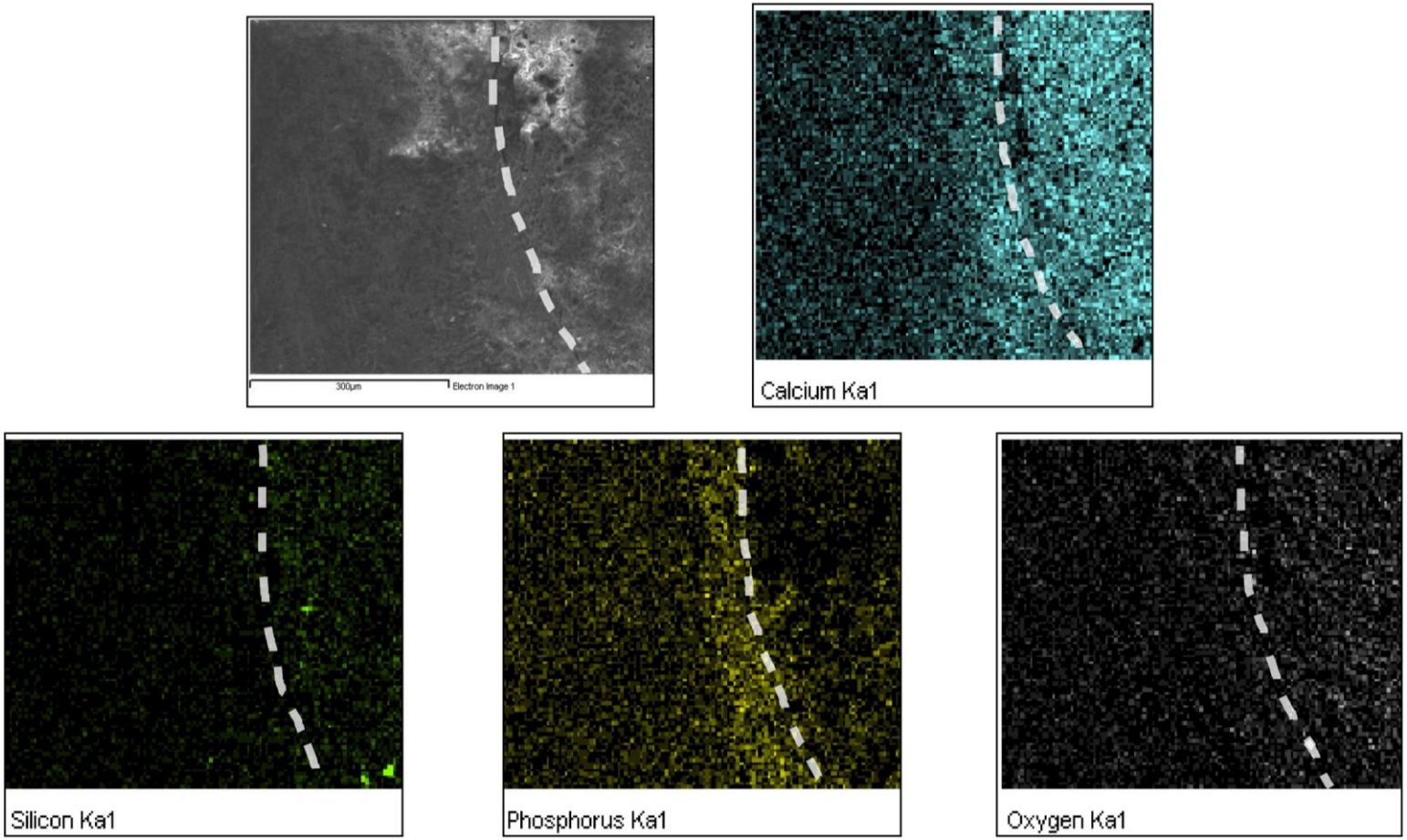

Figure 5.48. Mapping results for group MTA(W), mapping No. 4. Dotted line shows the approximate margin of the MTA.

CHAPTER SIX: DISCUSSION

6.1. FRACTURE RESISTANCE

6.1.1. Introduction

The main purpose of this research was to determine the fracture resistance of teeth with immature root apices with varying protocols. However, the methodologies employed in these studies showed great variation. In the present study an attempt was made to simulate clinical conditions.

Single-rooted human teeth with one root canal were used. After extraction all teeth were stored in water at room temperature until used in the investigation. Ideally the teeth should have been stored in Ca- and Mg-free PBS at 37^{o}C, but tooth collection for this work was undertaken in several centres with no access to a temperature controlled incubator.

It was not feasible to collect only immature teeth, therefore the single rooted teeth obtained were of different type, ages and stages of root development. This should be considered when interpreting the results of this study. They were however standardised according to the length and the size of the root canal. Careful allocation into the experimental groups according to the wall thickness ensured that tooth dimension would have a minimal effect on differences among the groups.

Teeth received endodontic treatment through an orthograde approach. An irrigation with 5.25% NaOCl and 10% CA was undertaken according to the currently recommended endodontic irrigation protocol (Basrani & Haapasalo 2012), although the volume of the irrigants and the time they were used for during the experiment were reduced compared with the clinical use. CA was used to remove a smear layer from the root canal wall.

The prepared samples were stored at 37ºC, in 100% relative humidity in closed containers with cotton wool rolls soaked in Ca- and Mg-free PBS which simulated tissue fluid. The storage conditions should be considered when interpreting the results of this study, because setting of MTA may be different in the presence of soft tissue fluid (Kim et al. 2012).

The roots were mounted for the fracture test to simulate as closely as possible the anatomical position in the mouth:

- Acrylic resin was used to simulate bone, as such a material has been shown to be able to reproduce the capacity of bone to withstand forces of mastication (Soares et al. 2005).
- A 2 mm gap between the CEJ and the top of the resin simulated the anatomical spacing found between the bone and the CEJ, as suggested by Wilkinson *et al.* (Wilkinson et al. 2007).
- The periodontal ligament was simulated, which was shown to play an important role in the fracture resistance tests by use of a polyether impression material to simulate the PDL (Soares et al. 2005).

- The specimens were loaded with the tip of a chisel at 130º to the long axis of the tooth in a lingual-labial direction, which simulated the inter-incisal angle in a Class I occlusal relationship in human. Such an approach has also been adopted previously (Hemalatha et al. 2009).
- The specimens were loaded to failure, this mechanical test was used because it simulated clinical conditions and could give clinically-important results.

When interpreting the results of fracture resistance studies it should be kept in mind that the experimental set-up is an over-simplification of the highly specialised periodontal support tissues and the physiological forces applied to the teeth. The method used did not faithfully replicate the typical clinical forces applied to teeth. The forces used in this study were compressive that were applied progressively at a constant rate until the sample fractured. This does not take place clinically. Instead, tooth fracture is more likely to be caused by a sudden impact or by prolonged fatigue forces following cracking of the tooth structure. In addition, it is likely to be applied at varying angles to the tooth structure. Many teeth may also have coronal tooth structure that may provide increased resistance to fracture.

Tooth substance

It was decided to use human teeth in the present study despite the difficulty in standardising parameters.

However, several *in vitro* studies used animal teeth, either sheep or bovine. A systematic review comparing human and bovine teeth for research found inconsistent data, concluding that morphology, chemical composition and physical properties between the two substrates may influence the results obtained from any experiment using bovine teeth (Yassen et al. 2011). The results of studies using ovine teeth (Andreasen et al.2002, Andreasen et al. 2006, Hatibovic-Kofman et al. 2008) should also be considered with caution for the same reason.

Storage media

Fracture resistance may be influenced by the storage media. In some studies teeth were stored in 0.5%-1% Chloramine-T (Andreasen et al. 2002, Rosenberg et al. 2007, Hatibovic-Kofman et al. 2008, Whitbeck et al. 2011) before testing. Chloramine-T is a biocide and an oxidizing agent which in the presence of water breaks down into sodium hypochlorite. This in turn may affect the mechanical properties of dentine by degradation of organic dentine components (Oyarzun et al. 2002). Other studies used 4% formal-saline (Rosenberg et al. 2007) for pre-experiment teeth storage, this may also have an adverse effect.

Storage in 100% relative humidity is commonly used as for experimental work of this type (Doyon et al. 2005, Rosenberg et al. 2007, Whitbeck et al. 2011, Zarei et al. 2013, EL-Ma'aita et al. 2014) other studies have used saline (, Doyon et al. 2005, Andreasen et al. 2006, Sahebi et al. 2010, Tuna et al. 2011), saline with 1% antibiotic (Hatibovic-Kofman et al. 2008), or samples submerged in CH (Grigoratos et al. 2001).

Storage temperature

Temperature of sample storage before the experiment started varied from storage in a freezer (Andreasen et al. 2006) and 4ºC (Rosenberg et al. 2007, Tuna et a. 2011), to room temperature (Andreasen et al. 2002).

In the literature 37ºC is commonly used as storage temperature for experimental work (Doyon et al. 2005, Rosenberg et al. 2007, Whitbeck et al. 2011, Milani et al. 2012, Zarei et al. 2013, EL-Ma'aitaet al. 2014). In other studies the samples were stored at 4ºC and 6ºC (Andreasen et al. 2006, Hatibovic-Kofman et al. 2008). Storage temperature should be considered when interpreting the findings when CH is used as an intracanal dressing, CH has increased solubility in water at lower temperature, rendering it more chemically active than at body temperature (Fava & Saunders 1999), thus causing more damage to the collagen network in dentine than at body temperature. The specimens in the present study were stored at 37ºC, in 100% relative humidity, similar conditions were used by Bayram & Bayram

(Bayram & Bayram 2016) and Milani *et al.* (Milani et al. 2012) who stored their samples in a sponge moistened with PBS at 37^0C and 100% humidity.

Irrigation

There is also variance in the irrigation protocols were used in other studies. Some do not have irrigation with sodium hypochlorite (Andreasen et al. 2002, Doyon et al. 2005, Andreasen et al. 2006, Hatibovic-Kofman et al. 2008, Twati et al. 2009, Sahebi et al. 2010, Whitbeck et al. 2011, Tuna et al. 2011) whilst other use irrigation with NaOCl at different concentrations (Grigoratos et al. 2001, Rosenberg et al. 2007, Tuna et al. 2011, Milani et al. 2012, EL-Ma'aita et al. 2014). In one study (Doyon et al. 2005) no irrigation was undertaken during root canal preparation, irrigation with 17% EDTA followed by 5.25 % NaOCl, followed by saline was carried out the end of the sample preparation. These protocols do not correspond with the contemporary practice and may have influenced fracture resistance. Bayram and Bayram (Bayram & Bayram 2016) used 5.25% NaOCl irrigation throughout the process of sample preparation and EDTA for the smear layer removal.

Tooth preparation

In the present study 10 mm long roots, with apices enlarged to size over 150 ISO were used, resembling immature roots.

In other studies the teeth have been prepared in different ways prior to testing:

- Mature roots with apices prepared to the size of 170 ISO (Milani et al. 2012, Bayram & Bayram 2016),
- Mature teeth with apices of size 50 ISO (EL-Ma'aita et al. 2014), or smaller (Rosenberg et al.2007, Whitbeck et al. 2011, Zarei et al.2013),
- Immature thin-walled teeth treated through retrograde approach, with CH placed in the pulp chamber (Andreasen et al. 2002, Andreasen et al. 2006, Hatibovic-Kofman et al. 2008, Tuna et al. 2011),
- Horizontally or vertically sectioned teeth (Grigoratos et al. 2001, Doyon et al. 2005, Twati et al. 2009, Sahebi et al. 2010).

Mechanical tests

In the present study all specimens were loaded to failure. Various mechanical tests have been previously used to test fracture resistance, these include:

- Load to failure (, Andreasen et al. 2002, Doyon et al. 2005, Andreasen et al. 2006, Rosenberg et al. 2007, Hatibovic-Kofman et al. 2008, Sahebi, et al. 2010, Tuna et al. 2011, Milani et al. 2012, Zarei et al. 2013, EL-Ma'aita et al. 2014, Bayram & Bayram 2016),
- Microtensile fracture strength (Rosenberg et al.2007),
- Shear test (White et al. 2002),
- Edge chipping test (Whitbeck et al. 2011).

Periodontal ligament simulation

A simulated periodontal ligament was used for all teeth in the present study, using a similar technique to that employed by Zarei *et al.* and EL-Ma'aita *et al.* (Zarei et al. 2013, EL-Ma'aita et al. 2014). Soares *et al.* (Soares et al. 2005) demonstrated the importance of the PDL simulation in fracture resistance tests. They showed that fracture occurred at different locations along the root surface when a PDL was present; when the samples were embedded directly in acrylic resin there were only cervical fractures.

Direction of force

In the studies using whole roots or teeth (Andreasen et al. 2002, Andreasen et al. 2006, Rosenberg et al. 2007, Hatibovic-Kofman et al. 2008, Zarei et

al. 2013, EL-Ma'aita et al. 2014) the samples were mounted using various protocols:

- In some studies the samples were mounted rigidly in plaster of Paris. A chisel was placed on the labial surface of the specimen, and parallel to it prior to axial loading being applied at a cross-head speed of 1 mm / min (Andreasen et al. 2002, Andreasen et al. 2006).
- The samples were mounted rigidly in resin, the chisel was placed on the labial surface of the specimen, and the load applied either axially (Hatibovic-Kofman et al. 2008), or perpendicular to the sample (Rosenberg et al. 2007, Tuna et al. 2011, Bayram & Bayram 2016) at a cross-head speed of 1 mm / min.
- The samples were mounted in acrylic resin with a simulated PDL (, Zarei et al. 2013, EL-Ma'aita et al. 2014). In one study the samples were loaded axially at a cross-head speed of 1 mm / min, in the second study no details were given of either direction of force or the cross-head speed (Zarei et al. 2013).

In the present study the specimens were loaded to destruction at 130ºC to the long axis of the tooth in a lingual-labial direction. A cross-head speed of 5 mm / min was chosen because some teeth failed to fracture during a pre-experimental mock testing when 1 mm / min cross-head speed was used. A similar direction of force and a cross-head speed was used in the study by Milani *et al.* (Milani et al. 2012) but in their study no attempt to simulate the PDL was undertaken.

Control groups

The majority of studies used a control group of teeth that received no treatment, but some studies did not (Rosenberg et al. 2007, Tuna et al. 2011). However, Andreasen *et al*. (Andreasen et al. 2002) used one set of controls from a previous study where the protocol for testing was different. In the present study three control groups were used: Irrigation only (stored for 4 weeks before testing), 2/52 CH and 12/52 CH.

There have only been two clinical studies investigating the fracture resistance of traumatized teeth that received long-term treatment with CH (Cvek 1992, Al-Jundi 2004), they reported a 40% and 32% incidence of root fracture, respectively. However confounding factors including the stage of root development, presence of cervical root resorption, a technique for apexification, and the type of restoration provided had not been considered.

6.1.2. Effect of CH on fracture resistance of human teeth

Fracture resistance of teeth treated with CH has been investigated using a load to failure test and whole roots or teeth in studies *in vitro* (Andreasen et al. 1990, Grigoratos et al. 2001, Andreasen et al. 2002, Doyon et al. 2005, Andreasen et al. 2006, Rosenberg et al. 2007, Hatibovic-Kofman et al. 2008, Twati et al. 2009, Zarei et al. 2013).

The results of the present study show that the use of calcium hydroxide as an interim dressing up to 12 weeks has no effect on fracture resistance of human teeth. This is in agreement with a study by Hatibovic-Kofman *et al*. and Whitbeck *et al.* (Hatibovic-Kofman et al. 2008, Whitbeck et al. 2011).

The results of the present study differed from some other studies *in vitro* (Andreasen et al. 2002, Andreasen et al. 2006, Rosenberg et al. 2007) but this may be explained by differences in the study design. Those differences include the use of non-human teeth, storage conditions, differences in sample preparation and in sample mounting. There were also differences in the control groups.

Kawamoto *et al.* (Kawamoto et al. 2008) suggested that the alkalinity of CH might lead to the breakdown of the inorganic dentine structure or the denaturing of the collagen network, resulting in poorer fracture resistance. Because the collagen fibrils are not readily accessible to CH, so time is required for CH to penetrate into and denature the collagen fibrils for such an effect to occur. Penetration of non-setting CH into the human dentine tubules was not detected after 1 month of dressing but could be seen after 3 and 6 months (Twati et al. 2009). This may explain why there was no difference in fracture resistance between groups Irrigation only and 2/52 CH and 12/52 CH (Table 5.11) observed in the present study.

6.1.3. Effect of MTA on fracture resistance of human teeth

Several studies have compared the fracture resistance of teeth restored with MTA mixed with water with teeth that received no treatment (Andreasen et al. 2006, Hatibovic-Kofman et al. 2008, Milani et al. 2012, EL-Ma'aita et al. 2014, Bayram & Bayram 2016).

Two of those studies had similar protocols (Andreasen et al. 2006, Hatibovic-Kofman et al. 2008). Immature sheep teeth were used, and treatment was undertaken by way of a retrograde approach. The entire root and the pulp chamber were filled with MTA. The teeth were stored at low 6ºC and 4ºC, respectively, in saline or saline mixed with 1% antibiotics for either 100 days (Andreasen et al. 2006) or 2 weeks, 2 months and 1 year (Hatibovic-Kofman et al. 2008). Andreasen *et al.* (Andreasen et al. 2006) found no statistically significant difference between untreated teeth and teeth restored with MTA. Hatibovic-Kofman *et al.* (Hatibovic-Kofman et al. 2008) found no difference between the groups after a 2-week and 2-month storage, but showed significant reinforcing effect of MTA on dentine after 1 year of storage.

Milani *et al.* (Milani et al. 2012) investigated fracture resistance of human teeth prepared to resemble immature teeth, with apices of size 170 ISO. The teeth received irrigation with 5.25% NaOCl and were obturated with MTA. The samples were stored for 6 months in a sponge moistened with phosphate-buffered saline. The roots were embedded rigidly in acrylic resin and loaded to fracture at 130º to the long axis of the tooth in a lingual-labial

direction, at a across-head speed of 5 mm / min. The results of fracture resistance test showed higher fracture resistance of MTA-filled teeth in comparison to untreated teeth ($P < 0.05$).

EL-Ma'aita *et al.* (EL-Ma'aita et al. 2014) used anterior human roots, prepared up to the size 50 ISO. 1% NaOCl was used for irrigation during the sample preparation, followed with sterile water. The coronal 2 mm of the root was left unfilled with MTA .The samples were stored at body temperature in synthetic body fluid for 48 h, 1 and 6 months. The roots were embedded in acrylic resin, the PDL was simulated using a light-body silicone impression material. The samples were loaded axially to fracture at a cross-head speed of 1 mm / min. The study found an increase in fracture resistance in teeth restored with MTA compared to untreated teeth after 1 and 6 months.

In the study by Bayram and Bayram (Bayram & Bayram 2016) human teeth were prepared up to the size 170 ISO. Samples received either no treatment or were obturated with MTA and stored at 37ºC and 100% relative humidity for 7 days. The samples were rigidly embedded in acrylic resin and loaded perpendicular to their the long axis at a cross-head speed of 5 mm / min. The study also found that the teeth obturated with MTA had higher resistance than the control.

The present study showed that fracture resistance of teeth treated with MTA mixed with water is significantly higher than the teeth that received irrigation

only ($P < 0.0001$). This is agreement with studies that used human teeth (Milani et al. 2012, EL-Ma'aita et al. 2014, Bayram & Bayram 2016).

Andreasen *et al.* (Andreasen et al. 2006) reported contrasting results, as well as Hatibovic-Kofman *et al.* (Hatibovic-Kofman et al. 2008) who reported no root strengthening effect of MTA mixed with water on ovine dentine after a 2-week and 2-month storage periods, but after a 1 year storage root strengthening effect of MTA mixed with water was detected. The difference in results could be because of the small sample numbers of sheep teeth and different protocols were employed in those studies. These included the use of ovine teeth, storage at low temperature, using a retrograde approach for treatment and differences in sample mounting.

Belli *et al.* (Belli et al. 2011) investigated distribution of functional stresses in a model of the maxillary central incisor filled with MTA and other materials and posts, using a 3-dimentional finite element stress analysis. They discovered that the interfaces of materials with different modules of elasticity represent the weak point of the restorative system. The MTA-treated model revealed that the cement kept the stress inside the material body and directed towards the root *via* the root canal, therefore, stress at the lingual cervical region decreased under loading. The authors suggested cervical crown fractures can be prevented if the root canal is filled with MTA.

No study that investigated fracture resistance of teeth restored with MTA mixed with PBS, against which the findings of the present study can be compared.

The present study showed that the fracture resistance of teeth restored with MTA mixed with PBS is significantly higher than the teeth that received irrigation only ($P < 0.01$) (Table 5.11).

Several studies have shown the bioactivity of MTA in the presence of phosphate-containing fluid manifested as the ability to produce carbonated apatite / hydroxyapatite crystals (Sarkar et al. 2005, Bozeman et al. 2006, Reyes-Carmona et al. 2009, Reyes-Carmona et al. 2010a, Reyes-Carmona et al. 2010b, Dreger et al. 2012). When MTA mixed with water was placed in the root canal, Ca^{2+} released during the hydration process of the cement diffused through the dentinal tubules, reacted with Ca-free and Mg-free PBS, and produced calcium phosphate (phosphate ions are absent in MTA, therefore precipitation of calcium phosphate can only occur in phosphate-containing fluid). Calcium phosphate incorporated other ions and matured into Ca-deficient β-type carbonated apatite. The mineral uptake caused by chemical and structural changes in dentine may result in increased physical strength of dentine (Han & Okiji 2011). When MTA is mixed with PBS there is plenty of phosphate ions available to form carbonated apatite. This can explain the improved fracture resistance in group MTA(PBS).

The Weibull analysis undertaken in the present study highlighted statistically significant ($P < 0.05$) lower dependability of MTA(W) group

versus MTA(PBS) (Table 5.13 and Figure 5.5), which means that teeth filled with MTA mixed with water were more prone to fracture at low values of applied stress than teeth filled with MTA mixed with PBS.

Han *et al.* (Han et al. 2010) undertook morphological and chemical analysis of precipitates of MTA stored in either distilled water or PBS after 1 and 14 days. They discovered that calcium hydroxide and calcium carbonate were formed on the surface of MTA when stored in distilled water, whereas amorphous calcium phosphate crystals were formed on the surface of cement immersed in PBS.

It has also been shown that when MTA was sealed with a wet cotton pellet for 72 hours it produced superficial precipitation, occluding or partially occluding dentinal tubules, with elemental composition similar to MTA. When MTA mixed with water was stored for 2 months in Ca- and Mg-free PBS, tag-like structures containing mainly Ca and P, were found deeper in the dentinal tubules (Reyes-Carmona et al. 2010a).

When MTA mixed with water is applied onto dentine its particles could enter into the dentinal tubules (Komabayashi & Spangberg 2008) and react with phosphate ions present there and available through diffusion (from PBS-soaked cotton wool rolls in this experiment, or soft tissue fluid *in vivo* for Ca^{2+} ions released during the hydration of the cement form carbonated apatite (Tay et al. 2007), which can increase the physical strength of dentine (Han & Okiji 2011). However, the biomineralization process depends of the availability of phosphate ions and may be less predictable (Reyes-Carmona

et al. 2010a, Han et al. 2010). This may explain the difference in the Weibull moduli between teeth restored with MTA mixed with PBS and water.

6.1.4. Effect of CH pre-medication on fracture resistance of teeth filled with MTA

Only one study investigated the influence of CH pre-treatment on fracture resistance of teeth filled with MTA mixed with water (Andreasen et al. 2006). The study compared fracture resistance of immature sheep incisor teeth obturated with MTA mixed with water with and without a 30 day pre-treatment with CH. The teeth received treatment through a retrograde approach and were stored in saline at 6ºC for 100 days. They found no significant difference between the groups ($P = 0.86$).

The results of the present study found there is no difference in fracture resistance between samples that received irrigation only and those that received a 2- and 12-week pre-treatment with CH and obturated with MTA mixed with water (Table 5.11).

The mean fracture resistance of teeth filled with MTA mixed with water recorded in the present study, was significantly higher comparing to teeth that received a 2-week CH pre-treatment ($P < 0.0001$) and a 12-week pre-treatment with CH ($P < 0.0001$) (Table 5.11). This is contrast with the findings of Andreasen *et al.* (Andreasen et al. 2006), this may be explained by differences in the study design.

The present study found that possible changes in the collagen fibrils of human dentine after a 2-week and 12-week exposure to OH^- ions from CH had no statistically significant impact on fracture resistance of human teeth (Paragraph 6.1.2) and that MTA mixed with water has an ability to strengthen human teeth (Paragraph 6.1.3). It could therefore be hypothesized, that Ca^{2+} and OH^- ions from CH could diffuse through the dentinal tubules and:

- precipitate as solid CH, according to the precipitation reaction:

  $Ca^{2+}(aq) + 2OH^-(aq) \rightarrow Ca(OH)_2(s)$

- when Ca^{2+} ions come into contact with carbon dioxide ions or carbonate ions in tissue, calcium carbonate is formed which has no biological properties (Estrela et al. 1999)

- or, in the presence of phosphate-containing fluid in the dentinal tubules, increased pH and Ca^{2+} concentration may have enhanced the super-saturation of phosphate-containing fluid and promote calcium phosphate precipitation.

Calcium carbonate and solid calcium hydroxide deposits could present a chemical and mechanical obstacle to MTA adaptation to the root canal wall (Stefopoulos et al. 2008). When MTA mixed with water is used after the CH dressing, there may be some unhydrated MTA (Saghiri et al. 2009) and there may be only minimal amount of phosphate-containing fluid in the dentine tubules or available through diffusion from the PBS used for storage. Therefore, element migration from the MTA into the dentine is not

possible (Paragraph 6.3.2) and it is likely that mostly solid calcium hydroxide and calcium carbonate were produced. This may explain why placement of MTA mixed with water did not improve the fracture resistance teeth that had been previously dressed with CH for 2 and 12 weeks (Table 5.10).

The present study also found that fracture resistance of teeth that received a 2-week CH pre-treatment and filled with MTA mixed with PBS is significantly higher than the teeth that received irrigation only, and not significantly different to the teeth that received no CH pre-treatment. The present study however, found that the strengthening effect is lost when a 12-week CH pre-medication is used.

No difference in fracture resistance between groups MTA(PBS) and 2/52 CH + MTA(PBS) could be explained by the fact that the 2-week contact of CH with dentine was not long enough for it to penetrate human dentine tubules and denature collagen matrix in dentine to any great extend, and the medicament could be almost completely removed by irrigation with 10% CA and 5.25% NaOCl (Zou et.al. 2010). Carbonated apatite could be formed on the surface of the dentinal tubules because plenty of PBS was available when the MTA was mixed with PBS.

It has been demonstrated that apatite formation by the MTA-PBS system deposited among collagen fibrils and triggered the formation of an interfacial layer with tag-like structures at the MTA-dentine interface. The

presence of an intact collagen network may therefore, play an important role in the formation of the tag-like structures (Reyes-Carmona et al. 2009).

It may be that after 12 week of CH treatment there is precipitate in the dentinal tubules (Twati et al. 2009), and the collagen network has been damaged so that the MTA mixed with PBS is not able to react with the root structure. The deposition of carbonated apatite may have balanced out the damage caused by the CH alkalinity, but was unable to change the physical properties of dentine enough to improve its fracture resistance.

6.1.5. Effect of MTA on fracture resistance of teeth treated with CH

Andreasen *et al.* (Andreasen et al. 2006) compared fracture resistance of immature sheep teeth treated with CH for 100 days and teeth pre-treated with CH for 30 days (CH group) and restored with MTA and stored for further 70 days (CH + MTA group). The mean fracture strength for the CH group (n=6) was 225.5 (± 78.84) MPa and (n=8) 326.7 (± 84.03) MPa for the CH + MTA group. There was no statistical analysis of those results done.

The present study found that fracture resistance of teeth that received a 2-week CH treatment is not significantly different to teeth that were additionally filled with MTA mixed with water (Table 5.11), but is significantly lower than if teeth were additionally filled with MTA mixed with PBS (Table 5.13 and figure 5.5).

The present study also found that the fracture resistance of teeth that received a 12-week dressing with CH is significantly higher than teeth that

were also filled with MTA mixed with either water ($P < 0.05$) (Table 5.11) or PBS ($P < 0.05$) (Table 5.13, Figure 5.4).

The higher fracture resistance in group 2/52 CH + MTA(PBS) than 2/52 CH may be explained by the fact that the storage period was not long enough for CH to penetrate deep into dentinal tubules (Twati et al. 2009), and medicament was likely to be completely removed by irrigation (Zou et.al. 2010). Furthermore, OH^- ions released from CH and MTA did not cause damage to the collagen fibrils in dentine significant enough to prevent formation of carbonated apatite in adjacent dentine when MTA mixed with PBS was placed in the root canal. Carbonated apatite formation was reported to be responsible for an increased physical strength of dentine (Han & Okiji 2011).

When the cement is mixed with water, the biomineralization process depends of the availability of phosphate ions in the dentinal fluids or through diffusion, and may be less predictable (Reyes-Carmona et al. 2010a, Han et al. 2010) which could explain lower fracture resistance of 2/52 CH + MTA(W) group versus 2/52 CH and 2/52 CH + MTA(PBS).

The weakening effect of MTA mixed with water on human dentine when used as a root filling after 12-week pre-treatment with CH compared with a 2-week pre-medication found in this study, could be explained by the more severe denaturing effect on the collagen fibrils by further exposure to OH^- produced during the hydration of MTA (Yaltirik et al. 2004, Sawyer et al. 2012), and released for extended periods (Fridland & Rosado 2003, Heward & Sedgley 2011, Leiendecker et al. 2012).

6.2. FRACTURE MODE

6.2.1. Effect of treatment on fracture mode

EL-Ma'aita *et al.* (EL-Ma'aita et al. 2014) investigated the distribution of the fracture mode of teeth filled with MTA and untreated teeth, stored at 37ºC in a synthetic body fluid. Two fracture modes were detected: a split vertical fracture that extended along the long axis of the root, and a comminuted fracture that had multiple planes and resulted in the root being broken into several fragments. The split fracture was the most commonly detected type in their control group and teeth filled with MTA and stored for 48 hr. Within the teeth filled with MTA and stored for 1 month the distribution was 50% vs 50%, but when teeth were stored for 6 months, the comminuted fracture type was more common (58%).

The results of the present study suggest that the type of treatment a tooth received may influence the type of fracture. In the present study a split was the most common type of fracture (Table 5.14). 78% of teeth in Irrigation only group suffered a split fracture. The comminuted root fracture was more common in groups: MTA(PBS) (41%), MTA(W) (44%), and 2/52 CH + MTA(PBS) (56%). The results of this study agree the results of EL-Ma'aita *et al.* (EL-Ma'aita et al. 2014). From these results, it is evident that some

treatment modalities can support the root and bond it until the force is high enough to fracture the tooth into several pieces.

The results of the present study showed also that type of treatment may have influence on the depth of root fracture. No other published study has investigated the influence of MTA and CH apexification on the depth of fracture of teeth.

In the present study 72.5% of all samples suffered deep fractures across the root (either into the cylinder or VRF) (Table 5.15), which clinically would be below the alveolar bone level. Those teeth could not have been saved, at least not without forced orthodontic extrusion or surgical crown lengthening which may have significant consequences, especially for young patients. In group Irrigation only 95% of teeth fractured below the level of the cylinder. In some groups, more samples fractured in the more favourable way - above the cylinder level: 29% of teeth in group MTA(PBS), 33% of teeth in groups 2/52 CH + MTA(W) and 2/52 CH + MTA(PBS), and 50% of teeth in group MTA(W).

6.2.2. Effect of force on fracture mode

EL-Ma'aita *et al.* (EL-Ma'aita et al. 2014) investigated the distribution of the fracture mode of teeth filled with MTA, obturated with gutta-percha and a sealer and untreated teeth, stored at 37ºC in a synthetic body fluid. Two fracture modes were detected: split and comminuted. The maximum force at

fracture was recorded for each root. The study showed that comminuted fracture occurred at higher forces, and the difference was statistically significant.

The results of the present study found no statistically significant difference between the mean F-max for the two fracture types (*P* = 0.7920) (Figure 5.9).

It is difficult to compare these results the experimental groups were different in each study.

It appears that factors other than the fracture force may predispose endodontically treated teeth to either a split or comminuted fracture. Those predisposing factors could be either iatrogenic (loss of structural tissue, effect of chemicals, intra-canal medications, restorations and restorative procedures) or non-iatrogenic (history of recurrent pathology, anatomical position of the tooth or effect of aging of dental tissues) (Kishen 2006).

The present study found also no statistically significant difference between the mean F-max between three fracture depths: above the level of the cylinder vs into the cylinder vs VRF.

Within the literature there was no study that investigated influence of fracture force on the level of root fracture

It appears that factors other than the fracture force may predispose endodontically treated teeth to fracture above and below the level of the cylinder.

6.3. CHANGES AT THE MTA-DENTINE INTERFACE

6.3.1. The SEM examination

Several studies have investigated the dentine-MTA interface of samples exposed to phosphate-containing fluid for 2 or 3 months using optical or scanning electron microscopy (Sarkar et al. 2005, Reyes-Carmona et al. 2009, Reyes-Carmona et al. 2010a, Reyes-Carmona et al. 2010b, Dreger et al. 2012). All those studies used human teeth, one of the studies was carried out *in vivo* on rats with dentine tubes filled with MTA implanted subcutaneously for 90 days. The sample preparation for the SEM testing was not described in any of the studies, except that they were coated with 300-Å gold layer and examined at various magnifications (15-3000 x). On examination it was possible to distinguish the cement, interfacial layer and dentine. The interfacial layer had tag-like structures entering dentinal tubules (Reyes-Carmona et al. 2009, Reyes-Carmona et al. 2010a, Reyes-Carmona et al. 2010b, Dreger et al. 2012).

In the present study, it was possible to distinguish a 50-200 μm interfacial layer in some samples of teeth filled with MTA mixed with PBS. No such

findings could be noted in groups filled with MTA mixed with water (Figures 5.9-5.14). This agrees with the findings of all previously cited studies (Reyes-Carmona et al. 2009, Reyes-Carmona et al. 2010a, Reyes-Carmona et al. 2010b, Dreger et al. 2012).

Han *et al.* (Han et al. 2010) when undertaking morphological and chemical analysis of precipitates of MTA stored in either distilled water or PBS after 1 and 14 days, discovered that calcium carbonate and calcium hydroxide were formed on the surface of MTA when stored in distilled water, whereas amorphous calcium phosphate crystals were formed on the surface of cement immersed in PBS. The hydrated MTA immersed in Ca- and Mg-free PBS was shown to produced more mineral precipitation than when MTA was sealed for 72 hours with a moist cotton pellet (Reyes-Carmona et al. 2010a). This could explain why no interfacial layer was detected in teeth filled with MTA mixed with water in the present study.

The presence of the interfacial layer between MTA (in groups filled with MTA mixed with PBS) and dentine is some proof of the bioactivity of the material, and the ability to produce mechanical and chemical bonding with dentine. This is responsible for the superior sealing ability (biological seal), and prevention of marginal leakage (Martin et al. 2007) and material displacement (Reyes-Carmona et al. 2010a). This also suggests that a primary monoblock can be achieved in a root canal, which it has been challenging in the past (Tay & Pashley 2007). Because MTA and dentine have similar muduli of elasticity, 15-30 GPa (Tay & Pashley 2007) and 18.6

GPa (Eskitascioglu et al. 2002) respectively, a mechanically homogenous unit can be formed in the root.

No inter-tubular mineralization could be seen on any of the samples in the present study. This could be because the samples were prepared differently for the SEM examination and because small number of samples were tested. In addition, the 4-week storage period may not have been not long enough to observe the tag-like structures, or there was not enough phosphate-containing fluid for the process to take place. The inter-tubular mineralization was previously shown to be present mostly in part of the root adjacent to the source of phosphate ions (Reyes-Carmona et al. 2010b). The main focus of the present study was the fracture resistance and an attempt to explain the results by element diffusion from the MTA into dentine. Therefore, only a few SEM micrographs were taken as an addition to the element mapping.

6.3.2. Element diffusion

Han and Okiji (Han & Okiji 2011) investigated the uptake of Ca and Si from white MTA by bovine dentine in the presence of Ca- and Mg-free PBS. After the root canal preparation the samples were stored for 1, 7, 30 or 90 days in Ca- and Mg-free PBS. Chemical component bulk analysis and element mapping for the 60-70 μm dentine adjacent to MTA was carried out using

SEM-Electron Probe Micro-analyser. The examination revealed Ca- and Si-rich dentine areas along the MTA-dentine interface (Figure 2.9).

Other studies (Sarkar et al. 2005, Reyes-Carmona et al. 2010a, Dreger et al. 2012) investigated the dentine-MTA interface of human teeth stored for 2 months or 90 days at 37ºC in Ca- and Mg-free PBS or implanted subcutaneously in rats (Dreger et al. 2012). After examination using SEM-EDX, the element composition of the interfacial (IF) dentine revealed:

- reduced amount of Si in IF layer than in MTA and the presence of P in IF layer (Sarkar et al. 2005),
- IF layer contained mainly Ca and P (Reyes-Carmona et al. 2010a),
- dentine, IF layer and inter-tubular mineralization showed similar composition, although dentine had reduced amount of Si (Dreger et al. 2012).

The element mapping undertaken in the present study showed Si, P, Ca and O element diffusion from the MTA apical plugs into dentine in groups: MTA(W), 2/52 CH+ MTA(PBS) and 12/52 CH+ MTA(PBS). The diffusion of Si only could be observed in MTA(PBS) group. No element diffusion could be detected in groups: Irrigation only, 2/52 CH, 12/52 CH, 2/52 CH + MTA(W) and 12/52 CH + MTA(W). The results of the present study agree with the findings of studies previously cited, confirming that dentine has the ability to uptake Ca, P and Si from MTA. The present study has also found

O diffusion into the IF layer. Increased amounts of Ca, P and O can be attributed to carbonate apatite formation in the dentine adjacent to the MTA.

The element diffusion, including P (Figure 5.48), was detected in the present work, also in samples of group MTA(W) that were not exposed to small amount of PBS from cotton wool rolls soaked in Ca- and Mg-free PBS. It could be hypothesized that:

- MTA particles entered into the dentinal tubules (Komabayashi & Spangberg 2008, Reyes-Carmona et al. 2010a) and
- Ca^{2+} released during hydration of the MTA reacted with phosphate ions (from tissue fluid in dentinal tubules or diffused from the wet cotton wool rolls) and formed some carbonated apatite (Tay et al. 2007), and/or
- Solid calcium hydroxide and calcium carbonate were formed when no phosphate ions were available (Estrela et al. 1999, Han et al. 2010).

In the majority of the samples in the present study, Si and O seemed to accumulate in the same area in dentine, and diffuse deeper into dentine than Ca. This may be explained by the fact that functional groups, such as Si-OH pre-existing on nano-porous calcium silicate hydrate gel structures (during the MTA hydration process) act as nucleation centres for apatite precipitation (Kokubo & Takadama 2006). The exact role of Si in hard tissue formation is unclear but Si is reported to induce remineralisation of demineralised dentine *in vitro* (Saito et al. 2003).

The diffusion of only Si into IF layer could be detected in group MTA(PBS). This may be because the movement of calcium is difficult to show using the SEM and elemental mapping, since Ca is present in both the material and dentine (Marciano et al. 2014), or because MTA was deposited in the dentinal tubules.

No element diffusion could be detected in groups 2/52 CH + MTA(W) and 12/52 CH + MTA(W) in the present study, and there was a 100 μm band of P-depleted dentine and Ca deposition around the MTA in 12/52 CH + MTA(W) group (Figures 5.17 and 5.18). It is likely that precipitate produced during CH pre-medication prevent element diffusion from the MTA. Even if these deposits could be removed by irrigation, there will be very little, if any phosphate ions available for Ca^{2+} ions released from MTA mixed with water to produce carbonated apatite. The lack of element migration from the MTA into the dentine in teeth that received CH pre-medication and filled with water in may be responsible for no root strengthening effect reported in the present study (Paragpaph 6.1.4).

CHAPTER SEVEN: CONCLUSIONS

7.1. LIMITATIONS OF THE PRESENT STUDY

- The teeth used were of different type, age, and stage of root development.
- The experimental set up is an over-simplification of the periradicular tissues with regard of soft tissue fluid exchange and the highly specialized periodontal support tissues. The method used did not faithfully replicate the typical clinical forces applied to teeth.
- Small number of samples were examined by the SEM and SEM-EDX.

7.2. CONCLUSIONS

The null hypothesis that placement of Mineral Trioxide Aggregate apical plug has no influence on the vertical root fracture of immature human teeth must be rejected because one-visit apexification with a 5 mm apical plug of MTA mixed with either water or Ca- and Mg-free PBS has been shown to significantly improve fracture resistance of those teeth.

The null hypothesis that treatment with a disinfecting dressing of calcium hydroxide before MTA apexification does not have an adverse effect on the fracture resistance of immature human teeth must be rejected, because 2- and 12-week calcium hydroxide pre-treatment has been shown to negate the fracture strengthening effect that MTA mixed with water would otherwise have had on those teeth. Calcium hydroxide pre-medication affected the fracture resistance of roots filled with MTA mixed with Ca- and Mg-free PBS in different way: root strengthening has been shown in teeth that received a 2-week treatment with CH but has not, when CH dressing was used for 12 weeks.

The null hypothesis that apexification with MTA mixed with Ca- and Mg-free PBS has the same effect on fracture resistance of immature human teeth as MTA mixed with water must also be rejected, because one-visit apexification with MTA mixed with Ca- and Mg-free PBS has been shown to be the most dependable treatment option to strengthen thin-walled teeth. A

significant improvement in the fracture resistance of human roots can be also achieved when a short-term (2 weeks) calcium hydroxide pre-treatment is used and roots are obturated with a 5 mm apical plug made with MTA mixed with Ca- and Mg-free PBS. No such effect has been shown when MTA mixed with water was used.

This study, with its limitations, has confirmed that:

1. CH dressing for up to 12 weeks has no negative effect on fracture resistance of human roots.
2. There is no significant change in fracture resistance of teeth that received a 2- and 12-week treatment with CH and were restored with MTA mixed with water, or treated with CH for 12 weeks, and restored with MTA mixed with Ca- and Mg-free PBS, in comparison with teeth that received no treatment.
3. One-visit apexification with MTA mixed with either water or Ca- and Mg-free PBS can significantly improve fracture resistance of human teeth. One-visit apexification with MTA mixed with Ca- and Mg-free PBS is the most dependable treatment option to strengthen thin-walled teeth.
4. If CH dressing is required for disinfection of a thin-walled tooth before apexification with MTA, it is better to use it short-term (2 weeks) and

obturate the root canal with MTA mixed with Ca- and Mg-free PBS. This will have a root strengthening effect on the human teeth.

5. Obturation with MTA apical plug has negative effect on fracture resistance of human roots treated with CH for extended periods (12 weeks).

6. The fracture force has no influence on the type or depth of root fracture in teeth treated with either CH or MTA apexification. The type of apexification treatment may have an influence on the depth and the type of root fracture.

7. MTA mixed with PBS can produce an interfacial layer between the cement and the dentine even in teeth that received pre-treatment with CH.

8. The element diffusion from MTA into dentine is possible when MTA is mixed with PBS, even if pre-treatment with CH was used. When MTA is mixed with water, CH pre-medication seemed to prevent the element migration to dentine.

7.3. SIGNIFICANCE TO CLINICAL PRACTICE

The results suggest that in clinical practice, subject to satisfactory clinical evaluation:

1. Calcium hydroxide can be used for up to 12 weeks, instead of 4 weeks as previously suggested (Andreasen et al. 2002) for root canal disinfection without a negative effect on fracture resistance of human teeth.
2. MTA mixed with water has no root strengthening effect on human teeth that received 2- or 12-week CH pre-treatment. The strengthening effect of MTA mixed with Ca- and Mg-free PBS on teeth that received a 12-week CH dressing was also not observed. Therefore, those treatment protocols should be avoided.
3. One-visit apexification with MTA mixed with Ca- and Mg-free PBS is the best treatment option with regard to improving fracture resistance of teeth.
4. If CH pre-medication is required, it should be done short-term (2 weeks) and the teeth are better restored with MTA mixed with Ca- and Mg-free PBS. This will have root strengthening effect.
5. It appears that factors other than the fracture force may predispose endodontically treated teeth to either a split or comminuted fracture, or has an influence on the depth of fracture. The type of apexification

may be one of the factors influancing the depth and the type of root fracture.

6. MTA mixed with Ca- and Mg-free PBS may produce an interfacial layer and mechanical and chemical bond with dentine and prevent bacterial leakage and displacement of the root filling.

7. The element diffusion from MTA may result is the creation of a mechanical and chemical seal at the MTA-dentine interface and prevent marginal leakage (Martin et al. 2007) and root filling displacement (Reyes-Carmona et al. 2010a). The lack of element diffusion in teeth that received CH pre-medication and restored with MTA mixed with water may explain the lack of root strengthening effect.

7.4. FUTURE RESEARCH SUGGESTIONS

- Changes to the protocol regarding use of MTA mixed with PBS e.g.
  - Irrigation of root canal with PBS
  - Placement of a cotton pellet moistened with PBS over the MTA
  - Different storage periods
  - Using human tissue fluid for storage
  - Obturation of the entire root with MTA,
- Finite elemental stress distribution of teeth filled with MTA mixed with PBS,
- Investigation of physical properties of dentine after treatment using different methods, e.g. hardness, modulus of elasticity, flexure strength,
- Hydration of MTA mixed with Ca- and Mg-free PBS,
- SEM and SEM-EDX analysis undertaken on larger number of samples.

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CHAPTER NINE: APPENDICES

APPENDIX I

East of Scotland Research Ethics Service *(EoSRES)*

NHS SCOTLAND
Research Ethics Service

TAyside medical Science Centre
Residency Block Level 3
George Pirie Way
Ninewells Hospital and Medical School

R Graham Chadwick
Professor of Operative Dentistry and Dental Materials Science
The Dental School
Park Place
Dundee
DD1 4HN

Date: 23 April 2014
Your Ref:
Our Ref: **CYA/AG/14/GAS/0046**
Enquiries to: Mrs Caroline Ackland
Direct Line: 01382 383839
Email: Caroline.ackland@nhs.net

Dear Graham

Project Title: Fracture resistance of tooth roots filled with bioceramic endodontic materials.

You have sought advice from the East of Scotland Research Ethics Service on the above project. This has been considered by the Scientific Officer and you are advised that, based on the submitted documentation (email correspondence and table below), it does not need NHS ethical review under the terms of the Governance Arrangements for Research Ethics Committees (A Harmonised Edition).

Document	Version	Date
Email	N/a	Various
Protocol	Not Specified	Not Specified

The advice is based on the following:

- *The project is limited to the use of previously collected, non-identifiable material consisting of, or including cells, in accordance with the terms of donor consent*

If the project is considered to be research you may require ethical approval as outlined in The Research Governance Framework for Health and Community Care. You may wish to contact your employer or professional body to arrange this.

For projects that are not research and will be conducted within the NHS you should contact the relevant local Quality Improvement Team(s) who will inform you of the relevant governance procedures required before the project commences.

This letter should not be interpreted as giving a form of ethical approval or any

endorsement of the project, but it may be provided to a journal or other body as evidence that NHS ethical approval is not required. However, if you, your sponsor/funder or any NHS organisation feels that the project requires ethical review by an NHS REC, please write setting out your reasons and we will be pleased to consider further. You should retain a copy of this letter with your project file as evidence that you have sought advice from the East Scotland Research Ethics Service.

Yours sincerely,

Caroline

Caroline Ackland
Scientific Officer and Manager
East of Scotland Research Ethics Service

APPENDIX II

TASC
TAyside medical Science Centre

KG/CF
Wednesday, 30 April 2014

Dear Dr. Chadwick

Sponsor R&D Reference Number: 2014DE04
Study Title: Fracture resistance of tooth roots filled with bioceramic materials.

I can confirm that this study is not subject to the Scottish Governement Health Department Research Governance Framework for Health and Community Care.

I note also that you have received a letter from East of Scotland Research Ethics Service confirming that an ethical review by them is not required. I can advise also that, as the study does not require the participation of humans, that review by the University of Dundee Research Ethics Committee is not required. As the research will not be conducted in or through the NHS, NHS Tayside R&D management approval is not required.

I would advise that you register this local tissue collection, if you have not already done so, with Tayside Tissue Bank.

You are now free to commence the study.

For your information TASC Standard Operating Procedures and guidelines are available at http://www.tasc-research.org.uk/_page.php?id=157.

Finally please contact me should you have any queries.

Dr. Catrina Forde
Senior Research Governance Manager

Signed for and on behalf of
the University of Dundee

APPENDIX III

Dental School

DUNDEE

Donor Information Sheet and Consent Form for the Collection, Storage and Use of Childrens' Extracted Teeth in Fracture Resistance Research Project

For many years, extracted teeth have been used in dental research. These teeth are **not** taken just so research can be carried out on them but are available because you have lost the tooth as part of your routine dental care. Very often teeth are removed because your mouth is crowded and braces are needed to straighten your teeth. Sometimes a number of teeth are lost because of dental decay or trauma. These extracted teeth are very valuable for researchers as we can use them to learn about many aspects of dental disease as well as new filling materials. We have used extracted teeth in many types of research in dentistry for at least 100 years and we hope that we can use your tooth to help us find out more so that dental treatments can be improved. We will not know which is yours so that nobody will know that you allowed us to use your tooth. However, we will tell people what our research results are so that your kindness can be shown in the scientific progress that we make.

This is why we are seeking your approval to use teeth that may have been removed during your dental care for dental research. There will be no pressure applied to you to give us the teeth and your dental care will continue regardless of your decision. The following conditions apply:

1. No tooth/teeth will be removed that is not necessary for your treatment.
2. The tooth/teeth will be not be identified in the future as having been given by you. This means that you will **not** be able to stop us using the tooth/teeth at a later date because we will not know which is/are your tooth/teeth.
3. The tooth/teeth will be stored securely in a locked cabinet in the Dundee Dental Hospital and School and destroyed once the research is completed

If you do not agree to your tooth/teeth being used for research in this way, this will in no way affect your treatment now or in the future.

I.....(PRINT NAME) have read the above and agree that any tooth removed in the course of my care, as a necessary part of dental treatment, may be used for dental research. I understand that this tooth will not have my name associated with it and will be stored in a locked cabinet in Dundee Dental Hospital and School. My identity will be protected at all times.

Signed..... Date .....
(Patient)

I.....(PRINT NAME) have read the above and agree that any tooth removed in the course of the dental care of the child named and for whom I have responsibility, as a necessary part of dental treatment, may be used for dental research. I understand that this tissue will be anonymised and stored in a locked cabinet in Dundee Dental Hospital and School and therefore the child's identity will be protected at all times

Signed..... Date .....
(Parent or Guardian)

I have explained the request for teeth for research purposes and answered the relevant questions asked by the patient/patient's parent or guardian.

Signed..... Date .....(Dental Surgeon/ Specialist nurse)

THE SIGNED FORM SHOULD BE RETAINED IN THE PATIENT'S CASE NOTES

APPENDIX IV

Dental School

DUNDEE

Donor Information Sheet and Consent Form
for the Collection, Storage and Use of Extracted Teeth in Dental Research

Researchers at the University of Dundee and in NHS Tayside are committed to ongoing medical and dental research aimed at understanding more about disease processes in order to improve the care and treatment of patients in the future. One of the most valuable resources for such research is extracted human teeth which are given as a gift by patients who are having teeth extracted as part of their dental care. Real progress in the medical and dental fields is achieved by patients and researchers working together in this way.

The tooth/teeth in question is/are removed as a necessary part of your care and **not taken** specifically for research purposes. However, before any research is carried out on a tooth that is gifted by you, it is **important** that you understand how the tooth/teeth might be used in research and give permission for that use.

We are therefore seeking permission to use teeth extracted in the course of your dental care for dental research. These teeth are used on the understanding that the following conditions apply:

1. No tooth/teeth will be removed that is not necessary for your treatment.
2. The tooth/teeth will be anonymised so that you cannot be identified in the future. This means that you will **not** be able to withdraw consent for the use of the tooth/teeth at a later date because we will not know which is/are your tooth/teeth.
3. The tooth/teeth will be stored securely in a registered tooth tissue bank in a locked cabinet in the Dundee Dental Hospital and School and destroyed once the research is completed.

If you do not agree to your tooth/teeth being used for research in this way, this will in no way affect your treatment now or in the future.

I.....(PRINT NAME) have read the above and agree that any tooth removed in the course of my care, as a necessary part of dental treatment, may be used for dental research. I understand that this tissue will be anonymised and stored in a locked cabinet in Dundee Dental Hospital and School and therefore my identity will be protected at all times.

Signed..... Date .....
(Patient)

I have explained the request for teeth for research purposes and answered the relevant questions asked by the patient.

Signed..... Date .....
(Dental Surgeon/ Specialist nurse)

THE SIGNED FORM SHOULD BE RETAINED IN THE PATIENT'S CASE NOTES